

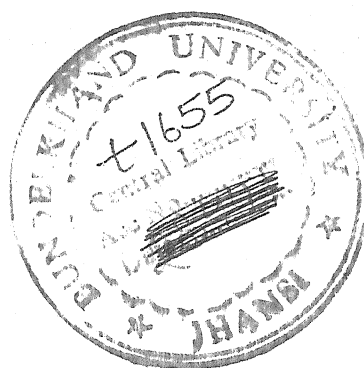
Chemistry of Natural Products From Some Indigenous Medicinal Plants

A THESIS

**SUBMITTED FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY IN CHEMISTRY
OF
BUNDELKHAND UNIVERSITY**

By

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DEDICATED

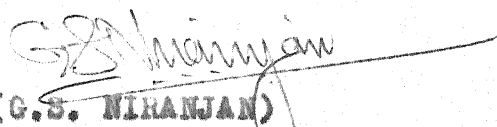
TO

MY PARENTS

SRI AND SMT. DR. B.N. SAXENA

CERTIFICATE

Certified that the thesis entitled "CHEMISTRY OF NATURAL PRODUCTS FROM SOME INDIGENEOUS MEDICINAL PLANTS" submitted by Sri Brijesh Chandra Sikroria, in fulfilment of the entire requirements for the Ph.D. degree of Bundelkhand University, embodies the record of his own investigation, carried out under my supervision and guidance and that this research work has not been submitted elsewhere for a degree. Sri Sikroria has put in more than 200 days attendance in the laboratory of Chemistry Department, Daya Nand Vedic Post-graduate College, ORAI of Bundelkhand University, Jhansi (U.P.).


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P R E F A C E

The present thesis entitled "Chemistry of Natural Products from some Indigenous Medicinal Plants" has been divided into five chapters and each chapter deals with different aspects of chemistry of natural products.

The first chapter describes the general survey on Indian Medicinal Plants covering the literature references up to 1979 and wide importance of natural products.

The Chapter II, forms the subject matter of chemical examination of stems of Plumbago zeylanica. Two quinones, one phytosterolin and an isoflavone were isolated and characterised from afore mentioned plant.

The Chapter III, the author has focussed on chemical studies of a steroid, a higher alcohol, two keto steroids and a chalcone, isolated from the roots of Tinospora cordifolia. The structures were determined on the basis of degradative, IR, UV, NMR and Mass spectral studies.

The Chapter IV describes the chemical examination of roots and stems of Eclipta alba. A sterol, two higher

(11)

alcohols and a triterpene were isolated from roots of Eclipta alba whereas an alkaloid - 'nicotine' was isolated from stems. Structures of the compounds were confirmed on the basis of degradative and spectral studies.

The Chapter V screened the pharmacological studies of the isolated compounds from stems of Plumbago zeylanica, roots of Tinospora cordifolia and stems of Eclipta alba for their antifertility, antibacterial and fungicidal activity.

While every due care has been taken to give proper credit to other authors in the literature, the author would like to apologize for any omissions which might have occurred due to an oversight or error in judgement.

The work presented in the thesis has been carried out in the Chemical Laboratory, Dayanand Vedic Post-graduate College, Orai, under the supervision of Dr. G.S. Niranjan, D.Phil., F.I.C.S., Department of Chemistry, Dayanand Vedic (P.G.) College, Orai.

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Brijesh Chandra Sikroria.
(BRIJESH CHANDRA SIKRORIA)

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CHAPTER - I

GENERAL SURVEY ON CHEMISTRY OF NATURAL PRODUCTS FROM SOME
INDIGENOUS MEDICINAL PLANTS

I.1 GENERAL SURVEY ON "CHEMISTRY OF NATURAL PRODUCTS FROM SOME INDIGENOUS MEDICINAL PLANTS"

Since the dawn of human creation, diseases have been persuing and plaguing mankind. They threatened his very existence. It was, therefore, natural for man from the earliest times to try to find out means to ward off diseases and to find cure for them. In his quest for substances having curative values, he made an elaborate study of plants in his own way and was able to recognise the curative values of different plants for various ailments and diseases. The medical science in its earliar stage naturally developed where these plants were the only medicine to be used. A continued search for medicinal plants during the last several centuries has given us a store of innumerable plants which are of great use in the treatment of diseases and promotion of health.

Medicinal properties of plants are due to the presence of definite chemical constituents in them. These medicinal plants also contain some physiologically inactive and toxic substances. Because of these, the use of medicinal plants in their natural state present difficulties. It is, therefore, necessary to isolate the physiologically active substances from plants and to discard the inactive and toxic substances. By chemical examination, it is possible

to find out the exact composition of the drug and to synthesize it; thus avoiding altogether dependence on plants. In many cases these physiologically active constituents of plants have provided the chemist with a clue to the preparation of synthetic drugs of even greater potency.

The other important field for research in phytochemistry lies in the investigation of mode of action of drugs in relation to their chemical structure and physiological activity. By the knowledge of mechanism of the drug action, it is possible to synthesize related new drugs which may possess more therapeutic value and less toxicity. A classical example to this is the development of aspirin, which is a synthetic drug modelled on the natural drug salicin and it is useful for the relief of rheumatic and neurologic pains. Again the knowledge about the mechanism of action of the cocaine, guided chemists to the synthesis of a wide range of new drugs, such as procaine and novacaine which possess local anesthetic properties.

There is unfortunately no single test for medicinal virtue in plants. A test for antibacterial activity for example, will not reveal the presence of substances having narcotic effects or action on heart. The drugs like hashish and opium are not distributed in the plant tissues uniformly.

Furthermore, all substances may not be present in the plant at all stages of its growth. Plants grown in different climatic conditions show differences in their chemical constitutions.

It is evident that the systematic chemical examination of plants for new drugs is a huge tedious business. Nevertheless, many notable successes already recorded, give ample encouragement for the continuation of the phytochemical work. Modern chemical synthesis of drugs is entirely based on empirical correlation of the known synthetic drugs and they are not always synthesized in imitation of known natural drugs. Therefore, modern synthesis has very limited use in the synthesis of new drugs. Plant and other natural sources can provide thousands of substances, which may not be provided by the chemists for years, possibly centuries to come. A few of these substances can reasonably be expected to be useful. Development of phytochemistry as a research branch in India is very recent, although plant extracts and their preparations have been used in India for the treatment of disease since antiquity. All this has been followed empirically without any background of systematic chemical and pharmacological examination of drugs.

The study of vegetable drugs, although one of the ancient branches of sciences, has still a great importance.

The phytochemical investigation is not only a potent source of new drugs, but also it does make an important contribution to the allied fields of science.

The systematic work in plant chemistry began with the advent of the use of physical and biochemical methods. Thus a large number of compounds were discovered and their structures were determined. Introduction of paper chromatography, column chromatography and thin layer chromatography¹ have given a big impetus to the phytochemical methods. All these helped the phytochemists by simplifying the tedious process of separation and purification.

With the discovery of spectroscopic methods like Ultraviolet², Infrared³ and Nuclear Magnetic Resonance⁴, the phytochemists are provided with the indispensable tool for the investigation of molecular structures of compounds available even in small quantity. Now it is necessary to investigate their physiological and pharmacological properties. Only after these investigations, the drug can be clinically standardized for treatment of diseases.

There are still a large number of plants which have not been investigated for their active principles. A bright future awaits for plant chemists investigating the promiscuous treasure of nature to alleviate the human sufferings.

The plant products obtained from the plants are generally classified into following groups :

- (1) Alkaloids, (2) Glycoside, (3) Saponins,
- (4) Terpenes and terpenoids, (5) Bitter principles,
- (6) Essential oils, (7) Fatty oils and waxes, (8) Lactones,
- (9) Resins and tannins, (10) Steroids and phytosterolins,
- (11) Phenolic compounds, (12) Organic acids, (13) Hydrocarbons,
- (14) Gums and mucilages, (15) Sugars,
- (16) Quinones, (17) Higher alcohols and ketones,
- (18) Colouring matters.

This classification is not rigid, in the sense that one compound may be said to belong to more than one group according to its molecular structure.

The plants investigated in the present thesis were found to contain compounds belonging to the following groups:

- I.2. Flavonoid
- I.3. Quinones
- I.4. Sterols and Phytosterolins.
- I.5. Higher alcohols
- I.6. Alkaloids
- I.7. Triterpenes

I.2. FLAVONOIDS

The term flavanoid covers a large number of naturally occurring pigments in which two benzene rings are linked by a propane bridge ($C_6-C-C-C_6$) except in isoflavones in which the arrangement is $C_6-C-C-C_6$. The flavonoids include chalcones, dihydrochalcones, auroncs, flavones, flavonols, isoflavones, anthocyanidins and leucoanthocyanidins.

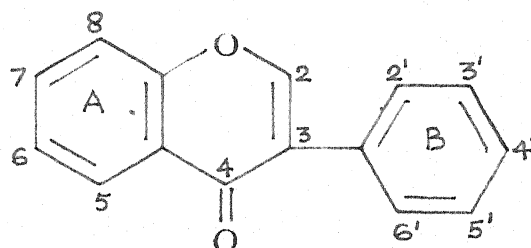
Flavonoids are present in plants as yellow pigments both in the free state as well in the form of glycosides. Disaccharides are occasionally present in the flavonoids which are so called Biosides.

Numerous physiological activities in plants have been attributed to the presence of flavonoids and related compounds. The small quantities of flavones may act as cardiac stimulents. Several flavonoid glycoside have been isolated from different plants sources and were found to be of great medicinal value.

In the present work, two flavonoid compounds, an isoflavone from stems of Plumbago zeylanica and a chalcone from the roots of Tinospora cordifolia, have been isolated and their structures were established by spectral data.

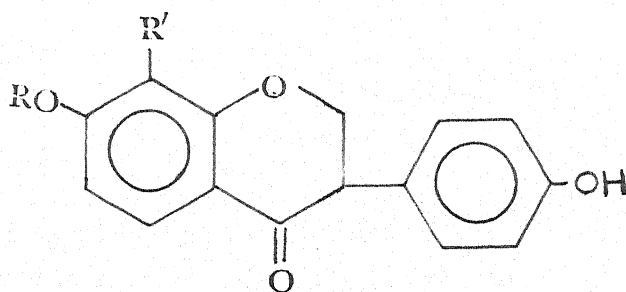
1.2.1. ISOFLAVONES

The isoflavones are naturally occurring colouring matters. They are 3-phenyl 4-chromone derivatives. The basic skeleton of isoflavone represented as follows :



Isoflavone Skeleton

Ononin was reported as the first isoflavonoid glycoside from the roots of *ononis spinosa*⁵. Later on plenty of isoflavone glycosides as well as free isoflavones have been reported. Shibata et al.⁶ isolated a number of isoflavonoids from the Pueraria roots. Among them, there were two known compounds, diadzein (Ia) and diadzin (Ib) which were also isolated from Soyabeans, while a new compound Puerarin (Ic) was found to be 8-glycosyl diadzein.



I

Ia, R=R'=H; Diadzein

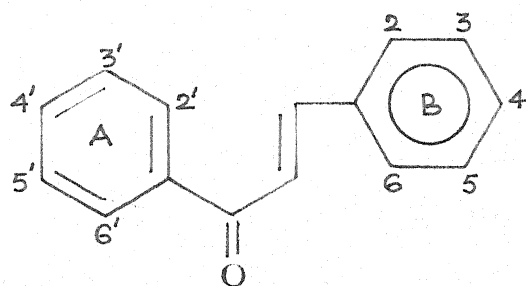
Ib, R=D-glucosyl, R'=H; Diadzin

Ic, R=H, R'=D-glucosyl, Puerarin.

Isoflavones have been reported to be highly medicinally important. Their importance in pharmacology is towards oestrogenic activity. Other isoflavones biochanin-A and prunetin were found to be oestrogenic. The isoflavone, diadzein showed antispasmodic action^{7,8}.

I.2.2. CHALCONE

Chalcones are naturally occurring open chain flavonoids in which two aromatic rings are joined to the terminals of, α , β -unsaturated system. Fundamentally they can be considered to be derivatives of phenyl styryl ketone. Naturally occurring chalcones are all hydroxylated to a greater or lesser extent. The numbering of the positions of substitution in the chalcone nucleus is reversed from that in other flavonoids. The general formula of chalcone is as follows :



Chalcone skeleton

Carthamine is the first natural chalcone which has been investigated by number of workers, but Perkin⁹

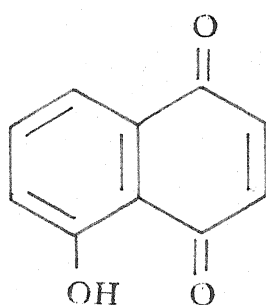
was the first to deal its structure. Kuroda¹⁰ showed that carthamine on treatment with dilute hydrochloric acid changes into an isomeric yellow compound isocarthamine. Later on, Seshadri¹¹ confirmed that carthamine is 2'-glucosidoxy-3':4:4':6'-tetrahydroxy chalcone by several chemical reactions. Another chalcone pedicin and its ether, pedicellin have been isolated from the leaves of Didymocarpus pedicellata¹². These two chalcones are major components and are accompanied by two quinochalcones, pedicinin and methyl pedicinin and a flavone, isopedicin.

Chalcones have also been reported to be active.

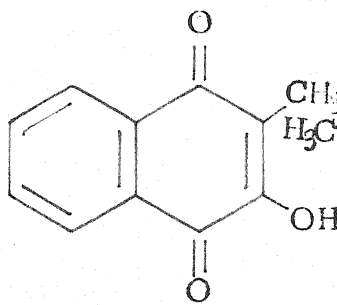
1.3. QUINONES

The quinones comprise a large group of natural pigments and are found mainly in plants, many of them have also been isolated from microorganisms and also from certain insects. In general, the quinones are yellow, red or brown in colour, but when present as salts of hydroxy quinones; their colours are purple, blue or green. The natural quinones play an important role in the oxidation-reduction processes of living matter and some of them have antibiotic properties.

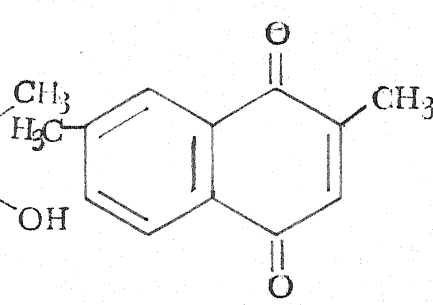
The naphthaquinones are mostly isolated from plants. Juglone, Phthical and Chinaphilin are the first reported naphthaquinones from the plants.



(Juglone)



(Phthical)



(Chimaphilin)

In the present work two compounds of naphthoquinone skeleton have been isolated from the stems of Plumbago zeylanica.

1.4. STEROLS AND STEROLINS

The sterols are polyphenolic and hydro-aromatic compounds having tetracyclic perhydro cyclopentene phenanthrene nuclei. Particularly phytosterols are widely distributed in nature and accompany fatty oils from which they can be separated as unsaponifiable matters. Although they occur in small amounts, they are of marked physiological importance. They occur invariably, where life exists and have profound importance in animal metabolism hormones, co-enzymes, bile acids and provitamin D. They are also occur in nature as glycosides.

The plants have a variety of closely related sterols called phytosterols. The best known phytosterols are stigmasterol, β -sitosterol, and ergosterol. Some steroids, recently isolated, are hirundigenin and

anhydro-hirundigenin from cynanchum grandifolium¹³, two withanolide steroids from Aenistus anstralis¹⁴, stigmasterol, β -sitosterol and an unidentified crystalline steroid from Ligustrum avalifolium¹⁵.

In the present work two new sterols were isolated from the roots of Tinospora cordifolia and their structures were established by degradative and spectral studies.

I.5. HIGHER ALCOHOLS

Higher alcohols are the long chain and high molecular weight containing organic compounds. They are the constituents of plant cuticle and waxes and sometime possess marked physiological activity. Long chain acetylenic alcohols are of great interest because of their pronounced pharmacological effects.

During last few decades, many higher alcohols were isolated from the plants. Ceryl alcohol from Ajuga bracteosa¹⁶ and from Clerodendrum phlomidis¹⁷, an alcohol from Melia azadirachta¹⁸ and a triterpene alcohol from Populus tremuloides¹⁹ were isolated. Jauhari et al.²⁰ and Murti et al.²¹ have also isolated and studied alcohols and waxes. Vanderburg and Wilder²² and Khalique and Kader²³ have studied the aromatic acids along with higher alcohols of Carnanba wax and the constituents of bees wax respectively. Recently, a higher alcohol has been isolated

Salmalia malabaricum²⁴.

Chibnall et al.²⁵ have reported that alcohols of higher molecular weight usually occur as the mixtures of alcohols having an even number of atoms from C₂₄ to C₃₆ and are difficult to separate. Spectral measurements have limited usefulness for characterization of these compounds. However, the unsaturation may be detected by UV spectra and nature of fundamental group such as hydroxyl and carboxyl by IR spectra. Gas chromatography has recently demonstrated its great usefulness in separating and identifying leaf wax hydrocarbons²⁶. Mass spectrum sometimes is very useful for locating the presence of functional groups and to measure the length of hydrocarbon chain while NMR spectrum is of limited applicability.

In the present thesis three higher alcohols have been reported and their structures were established by the use of UV, IR, NMR and Mass spectral data.

1.6. ALKALOIDS

Alkaloids are physiological active, nitrogenous basic compounds largely of plant origin. Accordingly, they often form salts with plant acids such as quinic or meconic acid. Some alkaloids are present in plants in free state or in combination with sugars (Solanine) or as esters (cocaine, atropine). Some alkaloids are present as quaternary salts.

Most of the alkaloids are colourless, crystalline compounds, a few, e.g., conine, nicotine and hygrine are liquids. Many alkaloids have bitter taste and quite a number of them possess curative properties. For example, morphine has a narcotic action, reserpine as tranquilizer, atropine as antispasmodic, cocaine, as local anesthetic and strychnine as nerve stimulant. The tobacco alkaloids are of the toxic nature and fatal for the man. They are also used as insecticides.

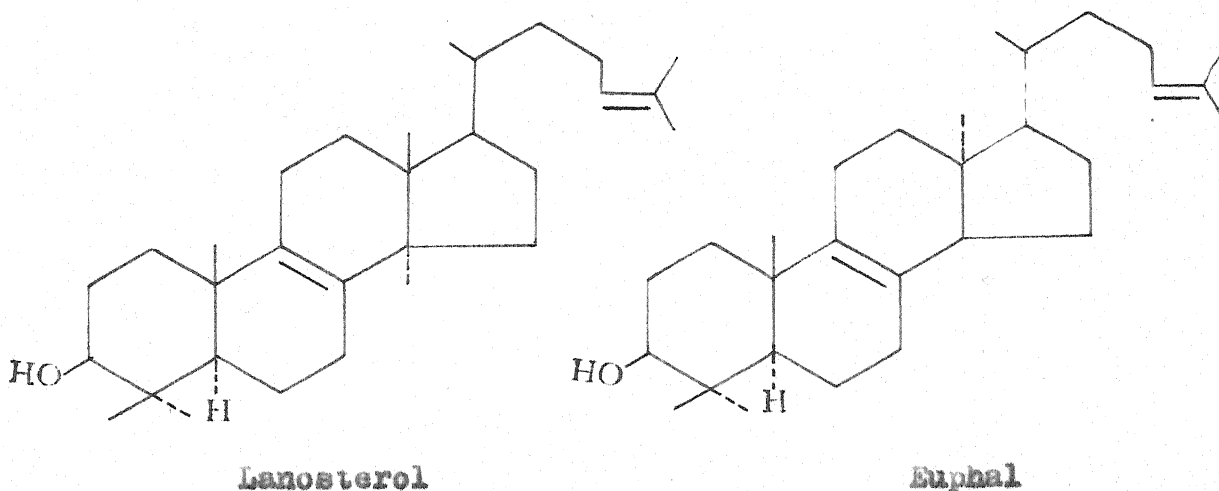
In the present work a compound of the class pyridine-pyrrolidine alkaloid, has been isolated from Eclipta alba. Many other alkaloids of this class have been reported in tobacco.

I.7. TRITERPENE

Triterpenoids are polycyclic, hydroaromatic, liposoluble alcohols. They are widely distributed in nature, occurring mainly in plants as esters and glycosides or in the free state. No triterpenoids has so far been reported to have a monocyclic or dicyclic structures. Tricyclic triterpenes are also rare and one important compound in this group is 'ambrein'. Most of the known triterpenoids have been found to have either tetracyclic or pentacyclic structures.

Tetraacyclic triterpenoids

They are of interest chiefly because of their resemblance and probable biogenetic relationship to the steroids. The two main families in this group of compounds to which most others are related can be represented in two substances lanosterol²⁷ and Euphal.



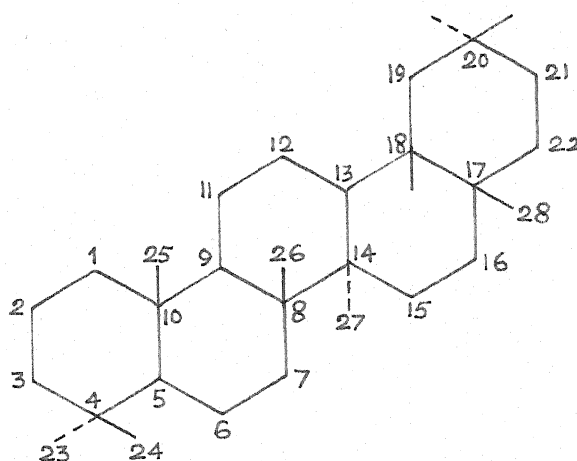
Agnosterol²⁸, eburicoic acid²⁹ and polypenenic acid³⁰ are the common members of these classes.

Pentacyclic triterpenes

They are found in plants both in free state and as glycosides (Saponins). The non-glycosidic triterpenoids are frequently found as excretions and cuticle where they may

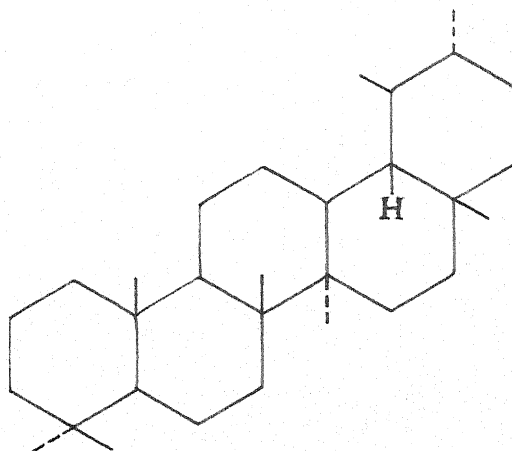
have protective or water proofing function. This class can be further divided into three main groups on the basis of chemical structures :

(i) Oleanane series :



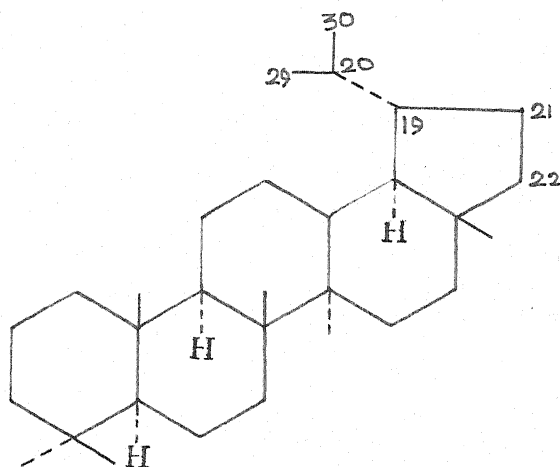
Oleanane

(ii) Ursane series :



Ursane

(iii) Lupane series :



Lupane

In general, pentacyclic triterpenoids are oxygenated at C_3 and in most cases the function is a β -hydroxyl group which is easily acylated. In few cases, an α -hydroxyl group as in boswellic acid or a ketonic group as in ieterogenin may occur. The nucleus may be saturated or may contain one or even more ethylenic linkage. A carboxylic group frequently occurs at C_{17} .

Physiological Activity of Triterpenoids

The following triterpens so far reported in the literature have been found to possess physiological action:

- 1) The cucurbitacins possessing a more striking activity are used in various traditional pharmacopoeia as vermifuges, narcotics, purgatives, emetics and for the treatment of

dropsy, dismenorrhoea and malaria. The substance cucurbitacin E (eleastrin) shows tumour inhibiting and anti cancer properties³¹.

2) A very interesting physiological activity has been observed in a family of triterpene antibiotics. Three such compounds namely helvolic acid, sepholysporin and fusidin have been reported. The last substance fusidin reported remarkably for its high antistaphylococcal activity, especially in combination with penicillin³¹.

3) Two new pentacyclic triterpenes isolated from *Agauria calicifolia* were found for curare-like activity³².

4) Ictrogenin³³ was found to be biological active, being responsible for "geet dikkop" in a complex syndrome including jaundice and photosensitivity in sheep. A probably related compound lantadene A, with the same physiological activity, has been reported by locula³⁴.

5) A pentacyclic triterpanic acid, Glycyrrhetic acid was found useful in the treatment of Addison's disease, asiaticoside, leprosy and tuberculosis.

6) The analogy of structure with steroids has prompted many attempts to prepare steroid hormone analogue with hormonal activity from some of the more accessible triterpenes, e.g.

- a) Petite et al.³⁵ synthesized 14 α -methyl testosterone from lanosterol.
- b) Fried³⁶ converted eburicoic acid into a number of oxygenated triterpenoid acid derivatives possessing progestational activity.
- c) Snatzke³⁷ prepared abeo and diabeo steroids from 8- α -lanosten-3- β -al acetate.

In the present thesis only one very well known triterpene β -amyrin has been isolated from the roots of Eolipta alba.

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CHAPTER - II

CHEMICAL EXAMINATION OF PLUMBAGO ZEYLANICA

The plant Plumbago zeylanica, commonly known as Chitraka, belongs to the family Plumbaginaceae.

Plumbago zeylanica is a 2-3 feet high pann perennial, straggling, diffuse, subscadent; stems clasping. Leaves are large, obovate elliptic, tapering to the short, somewhat clasping stalk. Inflorescence glandular, hairy, sticky, 4-12 inches long spikes; flowers bisexual, white; calyx persistent, tubular, glandular, 5-ribbed; corolla tube one inch long, slender; capsule in the persistent calyx, opening transversely near the base.

It is distributed throughout India and Pakistan.

The root is acrid, stimulant, diaphoretic, stomacnic, sialogogue, abortifacient and vesicant¹. In large doses it is narcotic and irritant. The root is given in dyspepsia, intermittent fevers, diarrhoea, piles, anasarea, skin diseases, rheumatism and paralysis; as an abortifacient it is used chiefly externally; the root bark is introduced into the mouth of womb.

As a Vesicant the root is valuable; its paste made with water or some bland oil is applied as an embrocation

over rheumatic and paralytic parts; the paste is also used over glandular tumours, buboes and abscesses; a paste made with salt and water is useful for obstinate skin diseases. The juice of the root, especially of the fresh root, is very acrid and blisters the skin.

The first examination of the roots of Plumbago zeylanica was made by M.C. Tumminkatti² and he reported a naphthoquinone, "Plumbagin". Later on G.S. Sindhu and AVB Sankaran³ examined the roots in detail. They reported plumbagin, 3-chloroplumbagin and 3,3' biplumbagin. Recently AVB Sankaran, A. Srinivasarao and G.S. Sindhu⁴ reported a new binaphthoquinone, "Chitranone" from the roots.

Literature survey reveals that no work has been done on the stems of this plant. Thus it was considered worthwhile to chemically examine the stems of Plumbago zeylanica.

EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS OF PLUMBAGO ZEYLANICA

The stems of Plumbago zeylanica were collected locally and identified for their authenticity in the Botany Department of D.V. College, Orai.

Dried and crushed stems (2 kg) were defatted with petroleum ether (60-80°) in a soxhlet extractor. The

defatted stems were extracted with chloroform several times. The extracts were combined and concentrated to a semisolid mass. Later on stems were extracted with ethanol (95%) in several lots. The total extract was concentrated to a viscous mass.

The semisolid mass refluxed with petroleum ether (40-60°) several times to remove fatty matters. The defatted semisolid was placed for steam distillation. The distillate was shaken with ether in a separating funnel several times. The ethereal layer was separated and the solvent was evaporated whereupon an orange yellow substance was obtained. The purity of the substance was tested on TLC which gave two spots. These two components were separated on silica gel G column eluting with petroleum ether : ethyl acetate (1:3 and 1:1) respectively. These eluates were distilled off and evaporated to dryness. The eluate I gave an orange yellow compound A and eluate II gave yellow compound B. The compound A was crystallised from chloroform and compound B from chloroform : benzene (1:1) and were kept in vacuum desiccator.

The viscous mass obtained with ethanol was refluxed with petroleum ether (40-60°) to remove fatty matters and resulting the still viscous mass was refluxed with solvent ether. The hot extract filtered whereupon a white shining substance 'C' settled down on cooling. It was separated

and crystallised from pyridine : ethanol (1:3) and kept in vacuum desiccator.

The residual viscous liquid was placed on silica gel G for TLC which gave three yellow spots on exposure with ammonia. Now these three components were chromatographed on silica gel G column prepared in petroleum ether (60-80°) and was eluted with following solvent systems -

- a) Petroleum ether
- b) Benzene
- c) Benzene : ethyl acetate
- d) Ethanol

The eluate C on evaporation gave a reddish brown compound 'D' which was crystallised from the benzene : ethyl acetate (1:1) mixture and kept in vacuum desiccator. The petroleum ether eluate failed to yield appreciable amount to examine in detail.

CHART - I

STEMS OF PLUMBAGO ZEYLANICA

Defatted with petroleum
ether (60-80°) in a
soxhlet for 20 hrs

Defatted material
Refluxed with
chloroform for 17 hrs

Extract

Extracted stems
Refluxed with ethanol
(95%) for 18 hrs.

Extract

Concentrated and refluxed
with pet. ether and
filtered

Residue

Steam
distillation

Filtrate

Extracted stems

Extract

Concentrated and
refluxed with pet.
ether and filtered

Filtrate

Residue

Extracted with hot
Et₂O and filtered

Residue

Chromatographed over
silica gel G column
& eluted with benzene:
ethyl acetate (1:1)
A reddish brown solid
COMPOUND 'D'

Ether extract
was cooled

A white mass
COMPOUND 'C'

Distillate

Chromatographed over
silica gel G column

Benzene : ethyl
acetate (7:3)

A yellow orange solid

COMPOUND 'A'

Benzene : ethyl
acetate (5:5)

A yellow solid

COMPOUND 'B'

Residue

SECTION - A

STRUCTURAL STUDY OF COMPOUND 'A'

An orange yellow crystalline compound 'A', m.p. 77°C was isolated from the stems of Plumbago zeylanica and purified as described on page 23. The elemental analysis of the compound gave molecular formula $C_{11}H_8O_3$, this was also in agreement with molecular ion peak at m/e 188 in its mass spectrum. It responded to colour reactions with ethyl malonate, ethyl cyanoacetate, ethyl acetoacetate and acetyl acetone, which showed the presence of perihydroxy naphthaquinone.

It decolourised potassium permanganate solution and bromine in carbontetrachloride; thereby suggesting the presence of unsaturation in the molecule. On bromination it formed a bromo derivative, mp 172°-3°C⁵. It was further confirmed by a prominent peak in the IR spectrum at 1660 cm^{-1} which can be attributed to α - β -unsaturated ketone or a quinone. The absorption bands above 212 nm in UV spectrum also favoured this fact.

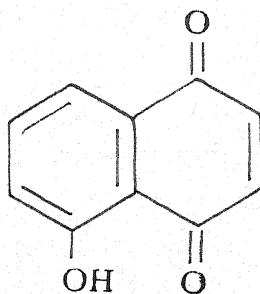
The presence of phenolic hydroxyl group was shown by peaks in the IR spectrum at 1400 cm^{-1} and 3455 cm^{-1} . The appearance of phenolic peak at 3455 cm^{-1} further showed that it was chelated or hydrogen bonded with some other part

of the molecule. It was further confirmed by a signal in NMR spectrum at δ 11.73 (Singlet)³ exchangeable with D₂O. On benzoylation it gave a benzoyl derivative, C₁₁H₇O CO C₆H₅ m.p. 145°C and on acetylation with acetic anhydride and pyridine, the compound yielded a monoacetate C₁₁H₇O CO CH₃ m.p. 117°C. All observations confirmed the presence of hydroxyl group.

The compound on deoxygenation by distillation with zinc dust gave naphthalene⁶ which showed that possibly the basic skeleton present in the compound was naphthaquinone.

The mass spectrum of the compound showed peaks at m/e 120, 92 and 63 indicated the presence of a hydroxyl group in the benzenoid ring⁷. In NMR spectrum, the proton signals for C-6H at δ 6.92-7.08 (quartet) and δ 7.33-7.46 (triplet) for C-7H and C-8H suggested that the hydroxyl group is present at C-5 in the benzenoid ring³.

From the foregoing discussions it is obvious that the hydroxyl group is chelated with anyone of the carbonyl groups of the naphthaquinone. The basic structure, therefore, may be shown as follows :

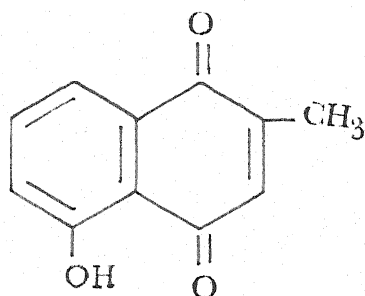


Thus all the three oxygen atoms, in the compound were accounted for and the remaining part to be fixed in this structure is $-\text{CH}_3$ which obviously must be present in the form of one methyl group attached at any one of the rest of the positions to the hydroxy naphthaquinone.

The compound was methylated using dimethylsulphate and alkali and the methylated products thus obtained were subjected to potassium permanganate oxidation, in this case 3-methoxy phthalic acid m.p. 173°C was obtained as the main product of oxidation. It clearly showed that the alkyl group was not attached to the benzene ring, but to the quinone ring. This is further supported by the peaks at 1120 cm^{-1} and 1040 cm^{-1} δ in IR spectrum of the compound 'A' which is characteristic of C-H in plane deformation in monosubstituted benzene ring. The signal at δ 2.02 (doublet) in the NMR spectrum revealed the presence of one methyl group at position-2.

On the basis of the above facts it may be concluded that the compound A belongs to naphthaquinone group having a hydroxyl group at position-5 and methyl group at position-2. Thus the compound 'A' was identified as 5-hydroxy-2-methyl-1,4 naphthaquinone (Plumbagin).

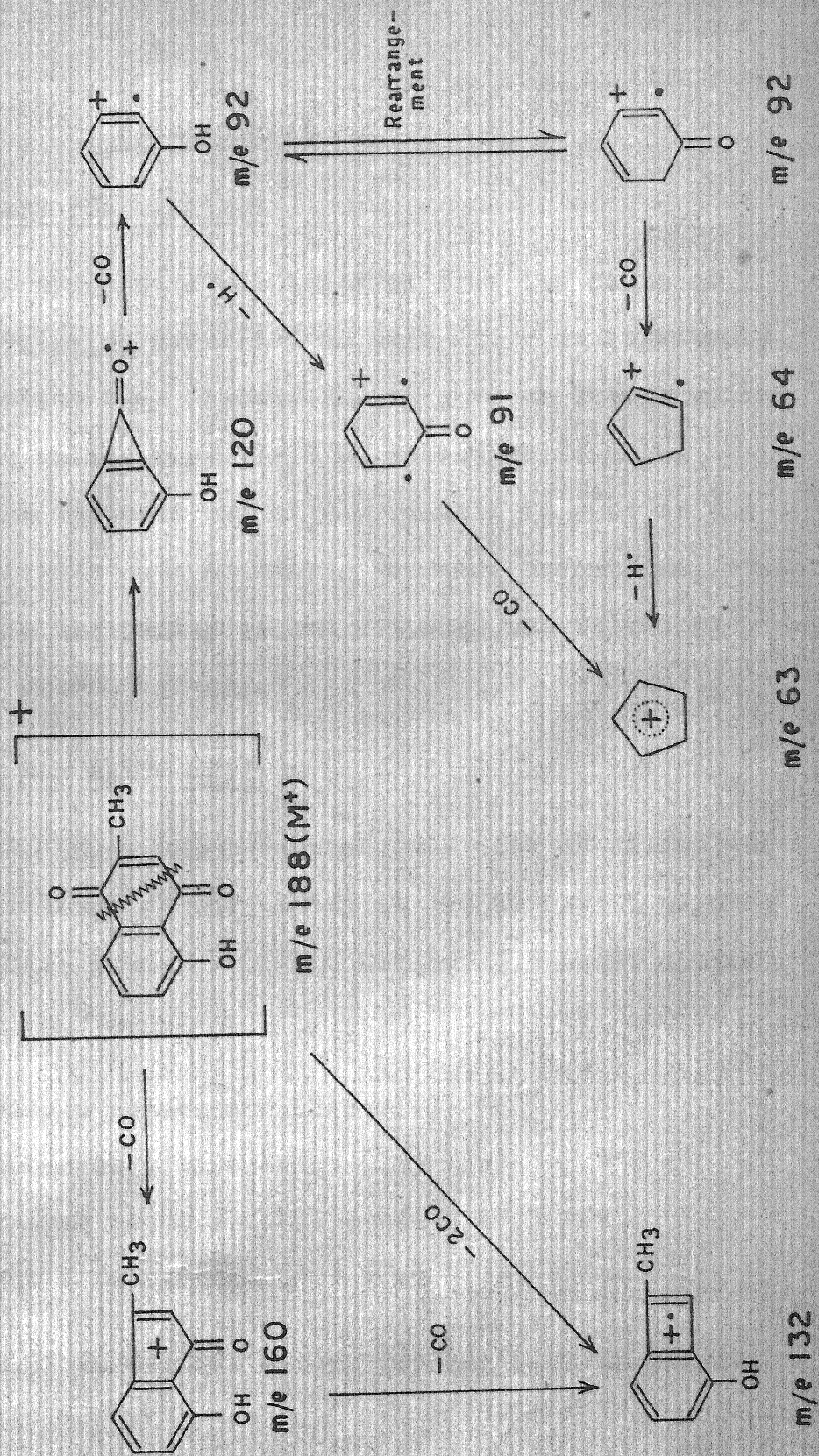
From the above data it is obvious that the structure for compound A may be written as



Finally the structure of compound A was confirmed by comparing the degradative chromatographic data with known Plumbagin^{7,9} reported in the literature¹⁰.

Further the structure of the compound A was confirmed by mass spectra which showed a characteristic peaks at M^+ m/e 188, 160, 132, 120, 92, 64, 63 (Scheme II.1). The quinone is first broken to its fragment m/e 120 which after co-elimination gave a fragment m/e 92. The fragment (m/e 92) showed fragmentation pattern similar to phenol. It gave characteristic peak at m/e 63.

SCHEME II. I. MASS



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound A was isolated from the stems of Plumbago zeylanica described on page 23 . On repeatedly crystallisation from chloroform, it gave an orange yellow crystalline solid, m.p. 77-78°C, molecular formula, $C_{11}H_8O_3$. The compound was highly soluble in most of the organic solvents such as ether, benzene, chloroform, acetone and ethanol. The purity of the compound was tested by thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plates by activating the plates at 100°C, for 15 minutes in an electric oven, using the following solvent systems for developing the plates.

- i) Benzene : chloroform (2:3 v/v)
- ii) Cyclohexane : chloroform (7:3 v/v)
- iii) Petroleum ether : ethyl acetate (7:3 v/v)
- iv) Hexane : chloroform (2:1 v/v)

R_f found 0.72 in solvent system (i) and 0.57 in solvent system (iii).

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{11}H_8O_3$</u>
C = 70.12%	C = 70.2 %
H = 4.4%	H = 4.26%
Mol.wt. = 188	Mol. wt.= 188
(By Mass Spectra)	

COLOUR REACTIONS¹¹

The compound gave following colour reactions :

- i) It gave blue colour changing to violet with ethyl malonate.
- ii) It gave violet colour changing to green with ethyl cyanoacetate.
- iii) It gave blue colour changing to violet red with ethyl acetoacetate.
- iv) It gave red colour changing to violet with acetyl acetone.
- v) It gave deep red colour with ethanolic ferric chloride.
- vi) It gave turbid solution, when treated with 2:4 dinitrophenyl hydrazine, but not a precipitate.
- vii) It dissolved in aqueous sodium hydroxide with crimson colour.
- viii) It decolourised bromine in carbon tetrachloride and potassium permanganate solution.

BENZOYLATION

Compound A (30 mg) was taken into a round bottom flask with 1 ml benzoyl chloride and 2 drops of pyridine and was kept for 48 hours. The reaction mixture was refluxed for 6-8 hours on a water bath. The contents were cooled and poured in ice cold water containing 2% aqueous sodium bicarbonate. A yellow mass was separated out which was filtered and washed with 2% sodium bicarbonate followed by distilled water. The mass was recrystallised from ethanol as light yellow solid, m.p. 145°C.

ACETYLATION

Compound A (30 mg) was taken in a 100 ml round bottom flask and 5 ml of acetic anhydride and 2 ml of pyridine were added to it. The reaction mixture was refluxed on waterbath for 3-4 hours and then poured in ice cold water and left overnight. It was filtered, washed well with water and was recrystallised from chloroform : methanol (1:1) mixture into yellow coloured needles m.p. 117-18°C.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{11}H_7OOCOCH_3$</u>
C = 67.51%	C = 67.83%
H = 4.4%	H = 4.35%

BROMINATION

The compound A (20 mg) was brominated with bromine in acetic acid and kept overnight. The contents were poured in ice cold water to get precipitate which was filtered, dried and recrystallised from ethanol into yellow needles, m.p. 172-73°C.

ZINC DUST DISTILLATION

The apparatus employed for distillation consisted of apyrex glass tube (20 cm long) open at both ends and having B₁₀ quick fit cone joint in the middle of the glass tube. One end of the tube was connected to a chain of three (50 ml) capacity flasks containing benzene and the last flask containing water was connected with an aspirator for suction. The other end of the tube was connected to an oxygen free nitrogen gas cylinder.

The compound (40 mg) were mixed with 500 mg of zinc dust and the mixture was taken in a 25 ml round bottom flask having a B₁₀ quickfit socket. The flask was attached to the B₁₀ quickfit cone of the tube. A slow current of nitrogen gas was passed from one end of the tube through the whole assembly of the apparatus, suctioned at the other end by a flow of water of the aspirator. After the air of the apparatus had been replaced by nitrogen gas, the flask containing the reaction mixture was heated at

high temperature. The distillate was received in the flask. Heating was discontinued when the evolution of white fumes, from the flask, had practically ceased. Flask was then disconnected.

The crude product obtained after distilling off the solvent, on crystallisation from hot ethanol was found to melt at 78-79°C which was not depressed by the addition of naphthalene.

METHYLATION

The compound (100 mg) were dissolved in acetone (6 ml), to this solution 3 ml of dimethyl sulphate and 5 g of potassium carbonate were added. The reaction mixture was heated for 3 hours under reflux. Potassium carbonate was filtered off and washed with acetone. Acetone filtrate was concentrated to a small volume and poured into ice cold water. The product so obtained was crystallised from ethanol.

POTASSIUM PERMANGANATE OXIDATION

The product obtained on methylation was subjected to potassium permanganate oxidation.

About 40 mg of the methylated product was dissolved in acetone and 10% aqueous potassium permanganate solution was added till its colour persisted. The mixture was

refluxed on water bath for 4 hours. The solution was cooled and acidified with concentrated hydrochloric acid.

The excess of manganese dioxide was removed by adding aqueous solution of sodium bisulphite. The solution extracted with ether and ether layer was washed thoroughly. The solution, on cooling gave a white precipitate which was filtered, crystallised and identified as 3-methoxy phthalic acid, m.p. 173°C (lit. m.p. $173-74^{\circ}$).

ULTRA-VIOLET SPECTRA

Spectral study of the compound was made using absolute ethanol as solvent using Perkin Elmer 202 spectrometer.

<u>Sample and Reagent</u>	<u>λ_{max}, nm</u>
Compound A + 95% ethanol	210, 255 sh, 267, 424.
Compound A + 95% ethanol + OH^-	211, 273, 575.

INFRA RED SPECTRA

The IR spectra (KBr) of the compound A was recorded on Perkin-Elmer Infra Cord spectrophotometer. It has prominent peaks at 3455, 2950, 1660, 1640, 1400, 1120, 1040 cm^{-1}

NMR SPECTRA

The NMR spectrum (CDCl_3) of the compound A, recorded on Varian A-60 D spectrometer, gave the following main signals, CDCl_3 (solvent), TMS as reference.

<u>Signals in δ value</u>	<u>Assignments</u>
2.02 (d) J = 1.5 c/s	Methyl group at position 2
6.58 (q) J = 1.5 c/s	3C-H
6.92-7.08 (q)	6C-H (one aromatic H)
7.33-7.46 (t)	7C-H and 8C-H (two aromatic H)
11.73 (s)	5C-OH (one perihydroxyl group)
exchangeable with D ₂ O	

MASS SPECTRA

The mass spectrum of the compound was recorded on Hitachi RMU-6E spectrometer which gave the following main signals.

Peaks at m/e 188, 160, 146, 132, 120, 118, 106, 96, 92, 82, 64, 63, 54.

SECTION - B

STRUCTURAL STUDY OF COMPOUND 'B'

The yellow crystalline compound B, m.p. 125-127°C was obtained from the stems of Plumbago zeylanica (page No. 23). The elemental analysis of the compound gave molecular formula $C_{19}H_7O_3Cl$, which was also in agreement with the molecular ion peak at m/e 222 in its mass spectrum. It responded to colour tests for the perihydroxy naphthaquinone¹¹.

It decolourised potassium permanganate solution and bromine in carbon tetrachloride; thereby suggesting the presence of unsaturation in the molecule. The prominent peaks at 1665 cm^{-1} in IR spectrum can be attributed for quinones with two C=O groups in the same ring. It was also confirmed by the absorption bands above 212 nm in the UV spectrum.

This quinone exhibited a molecular ion base peak at m/e 222 with a satellite peak at m/e 224. The relative intensities of $M+2$ to M as well as those of $M-CO$ peak ($m/e = 194$) to its satellite peak ($m/e = 196$) were 36% which suggested the presence of a chlorinated quinone¹².

The IR peaks at 3500 cm^{-1} and 1420 cm^{-1} showed the presence of chelated or hydrogen bonded phenolic hydroxyl group. It was further confirmed by a signal in NMR spectrum

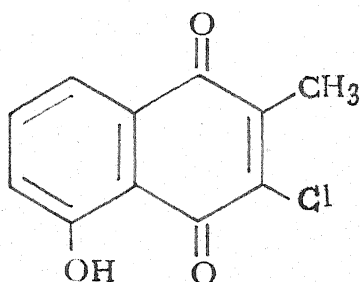
at δ 11.83 (singlet) exchangeable with D_2O . On benzylation it gave benzoyl derivative $C_{11}H_6OClOOC_6H_5$, m.p. 162-64°C.

The mass spectrum of the compound showed peaks at m/e 120, 92 and 63 indicated the presence of hydroxyl group in the benzene ring⁷. In NMR spectrum, the proton signals for C-6H at δ 7.30-7.47 (quartet), C-7H and C-8H at 7.70-7.80 suggested the position 5 for hydroxyl group³.

To ascertain the position of methyl group in quinone ring the methylated product of the compound was subjected to potassium permanganate oxidation whereupon 3-methoxy phthalic acid, m.p. 173°C was obtained as the main product of oxidation showing the presence of methyl group in quinone ring. It was further confirmed by the peaks at 1120 cm^{-1} and 1020 cm^{-1} in the IR spectrum of the compound. The signal δ 2.02 (singlet) in the NMR spectrum revealed the presence of one methyl group at position-2. It also confirmed the position of chlorine at position-3 which was further supported by the peaks m/e 166, 131 in mass spectrum of the compound.

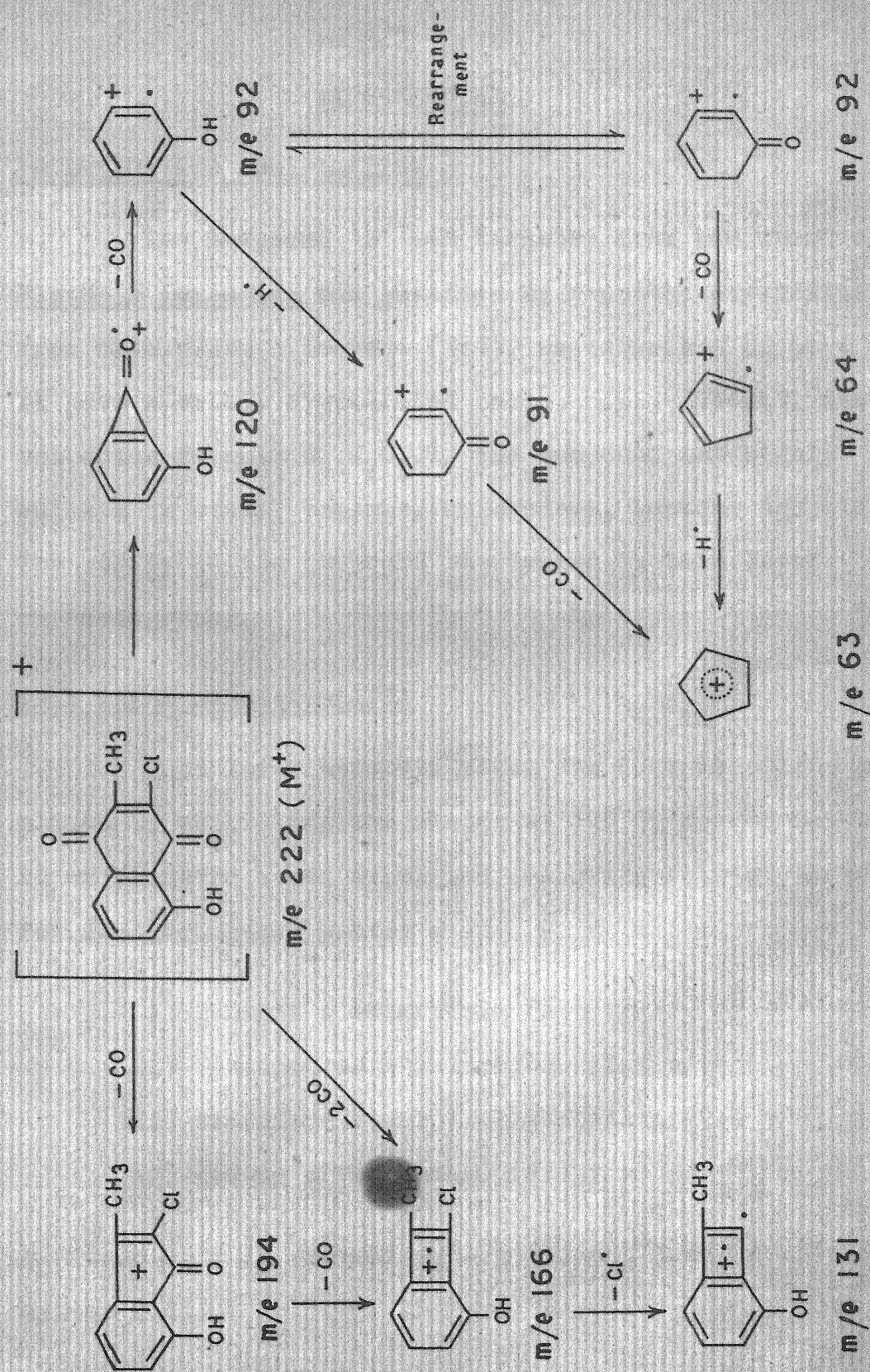
By comparing the spectral and chromatographic properties of this quinone with an authentic sample, it was identified as 3-chloroplumbagin (3-chloro-5-hydroxy-2-methyl-1, 4 naphthaquinone)¹³.

From the above discussion it is obvious that the structure for compound B may be written as :



The mass spectra of compound B (chloroplumbagin) showed a characteristic peaks at M^+ m/e 222, 194, 166, 131, 120, 92, 64, 63 (Scheme II.2). These fragments supported the above structure of the quinone, chloroplumbagin.

SCHEME II. 2. MASS FRAGMENTATION PATTERN OF COMPOUND - B



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound 'B' was isolated from the stems of Plumbago zeylanica and purified by repeated crystallisation from chloroform : benzene (1:1), as described on page 23. It gave a yellow crystalline solid, m.p., 125-26°, having molecular formula $C_{11}H_7O_3Cl$. The compound was highly soluble in ether, benzene, chloroform, acetone and ethanol. The purity of the compound was tested by thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plates by activating the plates at 100°C, for 15 minutes in an electric oven, using the following solvent systems for developing the plates :

- i) Benzene : chloroform (2:3 v/v)
- ii) Cyclohexane : chloroform (7:3 v/v)
- iii) Petroleum ether : ethylacetate (7:3 v/v)
- iv) Hexane : chloroform (2:1 v/v)

R_f found 0.82 in solvent system (i) and 0.63 in solvent system (iii).

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{11}H_7O_3Cl$</u>
C = 59.23%	C = 59.46%
H = 3.19%	H = 3.15%
Cl = 15.40%	Cl = 15.76%
Mol. wt. = 222 (Rast's method)	Mol. wt. = 222 (calculated)

COLOUR REACTIONS

- i) It gave blue colour changing to violet with ethyl malonate.
- ii) It gave blue violet colour changing to pale green with ethyl cyanoacetate.
- iii) It gave blue colour changing to red with ethyl acetoacetate.
- iv) It gave red colour changing to violet with acetyl acetone.
- v) It gave violet red colour with ethanolic ferric chloride.
- vi) It dissolved in aqueous sodium hydroxide with yellow colour.
- vii) It decolourised bromine in carbon tetrachloride and also aqueous solution of potassium permanganate.

BENZOYLATION

The compound (20 mg) was benzoylated by the usual method as described on page 33 . It gave a pale yellow substance which was crystallised from methanol, m.p. 162-64°C.

ACETYLATION

The compound B (30 mg) was acetylated with 5 ml of acetic anhydride and 2 ml of pyridine as usual given on page 33 . The acetylated compound was crystallised from methanol : chloroform (2:1) into yellow coloured solid m.p. 110-13°.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{11}H_6O_3Cl(COCH_3)$</u>
C = 59.15%	C = 59.09%
H = 3.46%	H = 3.41%
Cl = 13.13%	Cl = 13.26%

METHYLATION

The compound (20 mg) was dissolved in acetone (5 ml) to this solution was added 2 ml of dimethyl sulphate and 3 g of potassium carbonate. The reaction mixture was heated for 3 hours under reflux. Potassium carbonate was filtered off and washed with acetone. Acetone filtrate and washing were mixed together, concentrated to a small

volume and poured into ice cold water. The product obtained on crystallisation from ethanol gave a solid compound.

POTASSIUM PERMANGANATE OXIDATION

The product obtained by methylation was subjected to potassium permanganate oxidation, as described on page 35 .

The resulting compound was identified to be 3-methoxy phthalic acid by m.p. 173-74°C.

ULTRA VIOLET SPECTRA

Perkin Elmer 202 spectrometer was used for using absolute ethanol as solvent.

<u>Sample and Reagent</u>	<u>λ max, nm</u>
Compound B + 95% ethanol	216, 249, 280, 424

INFRA RED SPECTRA

The prominent peaks in the IR spectrum (KBr) of the compound B recorded on Perkin-Elmer Infra cord spectrophotometer are at 3500, 2930, 1665, 1635, 1420, 1120, 1020, 780 cm^{-1} .

NMR SPECTRA

The NMR spectrum (CDCl_3) of the compound B was recorded by Varian A-60 D spectrometer in CDCl_3 and TMS reference.

Signals in δ value

Assignment

2.02 (s)	quinone methyl group at position-2.
7.30-7.47 (q)	One aromatic H (6C- H)
7.70-7.80 (t)	Two aromatic H (7C- H and 8C- H)
11.83 (s)	One perihydroxyl group
exchangeable with D ₂ O	(5C-OH).

MASS SPECTRUM

The mass spectrum of the compound B was recorded on Hitachi RMU - 6E spectrometer, the following main peaks M^+ m/e 222 (satellite peak $M^+ + 2 = 224$), 194, (Satellite peak - 196), 187, 179, 166, 152, 151, 131, 120, 116, 92, 90, 69, 64, 63.

SECTION - C

CHEMICAL STUDY OF THE COMPOUND 'C'

The white crystalline compound C, m.p. 286-88°C, $[\alpha]_D^{30} + 128^\circ$ in pyridine, was isolated and purified from Plumbago zeylanica as described on page 23. The compound was insoluble in most of the organic solvents but highly soluble in pyridine. The elemental analysis gave molecular formula, $C_{35}H_{60}O_6$.

The compound C responded Liebermann-Burchard reaction^{14a} for sterols whereupon violet colour changing to blue green was obtained. It also gave other reactions for sterols.

Acid hydrolysis of the compound and subsequent examination of the hydrolysate by paper chromatography showed the presence of glucose which was confirmed by co-chromatography with an authentic sample of glucose and finally by the preparation of its osazone, m.p. 203-204°C.

The aglycone was obtained on shaking the hydrolysed product with ether. It was recrystallised from chloroform : methanol mixture into white flakes, m.p. 134-36°C,

$[\alpha]_D^{30} + 36^\circ$. The aglycone was highly soluble in petroleum ether, ether, benzene, chloroform, pyridine and carbon tetrachloride, sparingly soluble in acetone, ethanol and

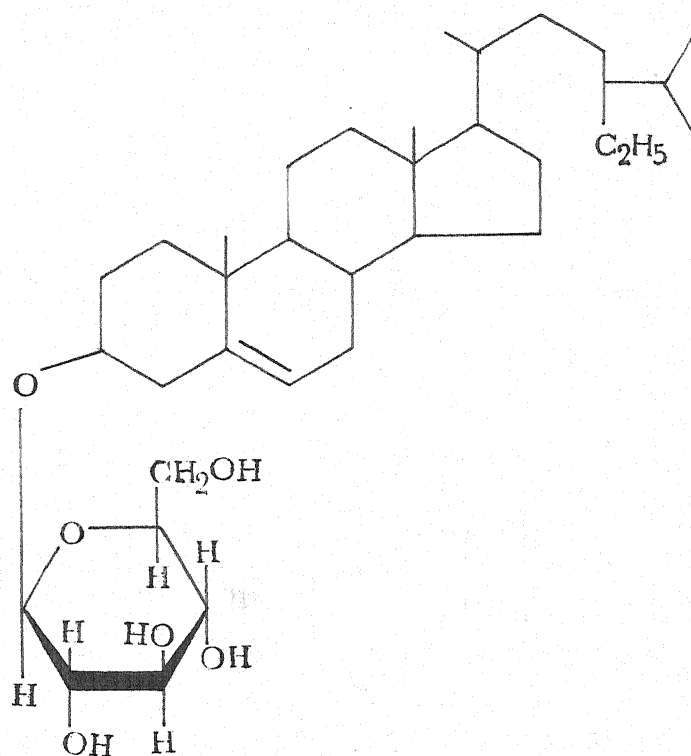
methanol. It also gave Liebermann Burchard reaction^{14a} with the same colour changes as were obtained with the glycoside. It gave a yellow colour soon changing to deep red with concentrated sulphuric acid^{14b} and a violet colour rapidly changing to blue with trichloro acetic acid. The solid compound gave a deep red colour with Noller's reagent^{14c}. On acetylation, the aglycone yielded an acetyl derivative, m.p. 124-25°C, $[\alpha]_D^{30}$ -37° in chloroform. Thus, the aglycone was found identical with β -sitosterol. It was finally confirmed by mixed m.p. with the authentic sample of β -sitosterol and superimposition of IR (Fig. III.1).

The methylation of the compound was first carried out with dimethyl sulphate and anhydrous potassium carbonate in dry acetone followed by methylation with methyl iodide and silver oxide. The methylated product was first hydrolysed with aqueous hydrochloric acid on boiling water bath followed by neutralisation with silver carbonate. The hydrolysate was examined by paper chromatography whereupon a single spot identified to be 2,3,4,6-tetra-*o*-methyl D-glucose was obtained. Thus it is clear that glucose moiety in the phytosterolin has free hydroxyl group at position 2,3,4 and 6.

The nature of sugar as to whether it is a mono-saccharide or oligosaccharide chain was determined by periodate oxidation of the compound C which showed the liberation of one mole of formic acid and consumption of

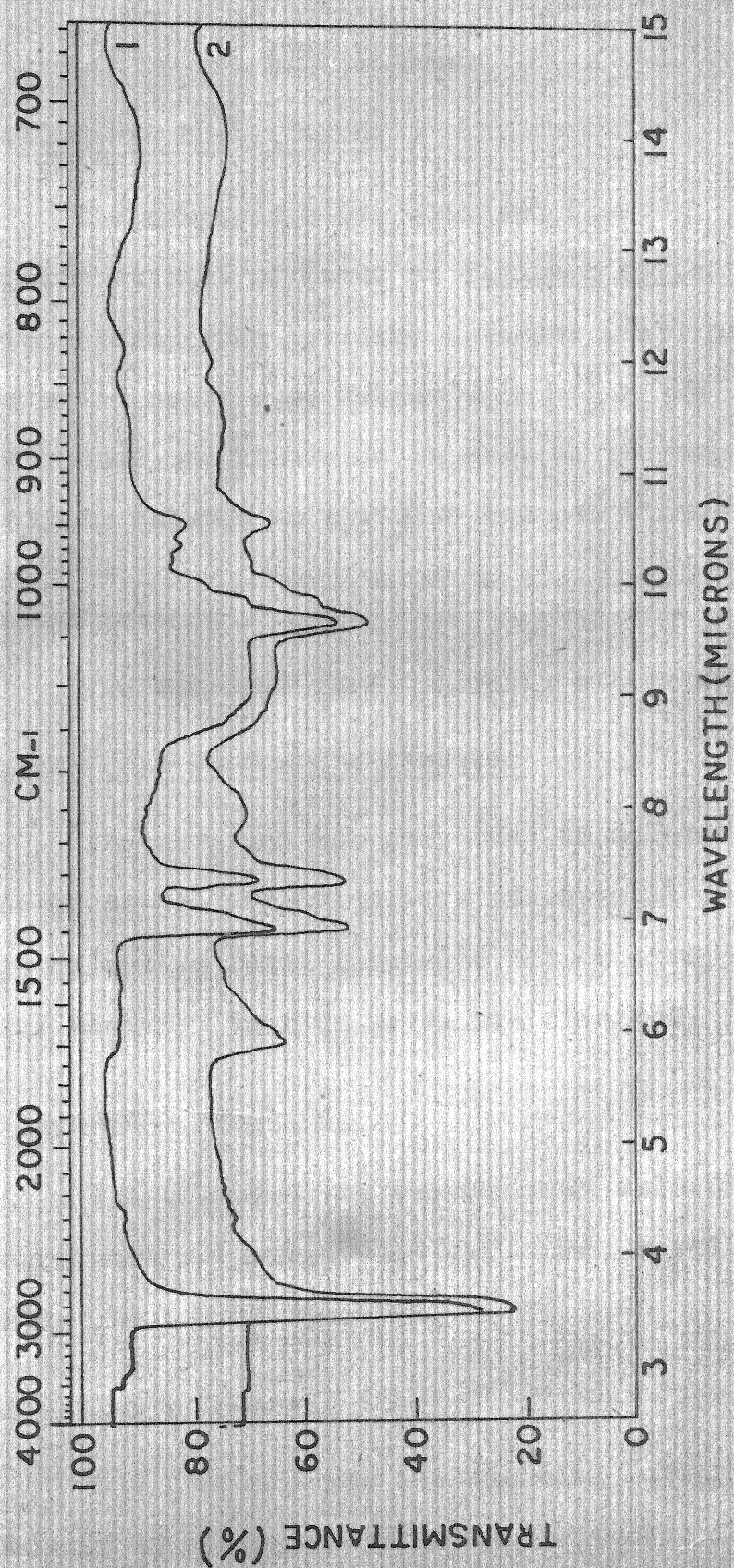
two molecules of metaperiodate per mole of the compound. Both of these results indicate that the compound C contain only one molecule of glucose in the glycosidic linkage. The optical rotation of the phytosterolin, its acetyl derivative and methylated compound suggested that the glucose is attached with β linkage which was further confirmed by the enzymatic hydrolysis with emulsin.

From the above discussion it is clear that the structure for phytosterolin may be as follows :



The nomenclature for the phytosterolin may be written as Δ^5 - stigmastene-3-ol- β -glucopyranoside.

FIG. II. I. INFRA RED (KBr) SPECTRUM OF COMPOUND - C



- 1. AUTHENTIC SAMPLE : β - SITOSTEROL
- 2. EXAMINED SAMPLE : β - SITOSTEROL

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound C was obtained (page 23) from the ethanolic extract of stems of Plumbago zeylanica and was recrystallised from pyridin; methanol (1:3) mixture into white solid mass, m.p. 287-88°C, $[\alpha]_D^{30} + 128^\circ$ in pyridin. The compound was insoluble in most of the organic solvents but highly soluble in pyridine and hot ethanol and methanol.

COLOUR REACTIONS

The compound gave following colour reactions :

i) Liebermann-Burchard's reaction

The compound was suspended in chloroform and a few drops of acetic anhydride were added to it followed by 2-3 drops of concentrated sulphuric acid whereupon a violet colour changing to blue green was obtained.

ii) Salkowski Reaction

The compound was suspended in chloroform and concentrated sulphuric acid was added, a yellow colour changing to red is observed.

iii) Noller's Reaction

The compound was treated with Noller's reagent (prepared by adding 0.01% stannic chloride in pure thionyl chloride), a deep red colour was obtained.

ACETYLATION OF THE COMPOUND 'C'

Compound C (50 mg) was taken in 10 ml of acetic anhydride and 1.5 g of fused sodium acetate was added to it. The reaction mixture was heated at 135-50°C for 8 hours and poured in ice water and left overnight. It was filtered, washed and dried. This mass was recrystallised from chloroform : methanol mixture (1:1) into light cream coloured flakes, m.p. 138-40°C, $[\alpha]_D^{30} + 120^\circ$ in chloroform

THIN LAYER CHROMATOGRAPHY OF ACETYLATED DERIVATIVE

Acetyl derivative in chloroform was applied on thin-layer plates made from silica gel G. They were developed with benzene : methanol mixture (85:15), sprayed with 50% sulphuric acid and heated at 120°C for about half an hour, whereupon a single spot was obtained in each case showing the purity of the acetylated product, therefore, the original compound was also pure.

HYDROLYSIS OF THE COMPOUND C

The compound C (100 mg) was hydrolysed with 8% hydrochloric acid in methanol for 10 hours on a boiling water bath. The hydrolysate was extracted well with ether. The aqueous solution obtained after the ether extraction was neutralised with silver carbonate and filtered.

IDENTIFICATION OF THE SUGAR BY PAPER CHROMATOGRAPHY

The aqueous solution obtained after neutralisation and filtration, was concentrated and examined by paper chromatography with the known samples of sugars as reference on Whatmann No. 1 filter paper in butanol : acetic acid : water (4:1:5 v/v) system as developing solvent. The paper chromatogram was air dried and sprayed with aniline hydrogen phthalate followed by heating in oven at 120°C for half an hour when a single spot corresponding to the mobility of glucose (R_f 0.18) was obtained. The sugar so obtained was thus confirmed to be glucose. Its osazone was also prepared in usual manner, m.p. 202-04°C.

IDENTIFICATION OF THE AGLYCONE

The compound obtained from etherial layer was recrystallised from chloroform : methanol (1:1) into white flakes, m.p. 134-36°C, $[\alpha]_D^{30} - 36^\circ$ in chloroform. It was readily soluble in ether, benzene, chloroform and carbon tetrachloride, but in ethanol and methanol on heating only. It gave Liebermann-Burchard reaction for sterols. It gave a yellow colour rapidly changing to deep red with concentrated sulphuric acid and a violet colour rapidly changing to blue with trichloroacetic acid. The solid compound gave deep red colour with thionyl chloride.

ACETYLATION OF AGLYCONE

The aglycone was acetylated with acetic anhydride and fused sodium acetate as usual, described on page 50 and recrystallised from chloroform : methanol (1:1) mixture into white flakes, m.p., 124-125°C. $[\alpha]_D^{30} -37^\circ$.

METHYLATION OF THE COMPOUND C

Compound C (100 mg) was taken in 25 ml of anhydrous acetone and to it 4 g of anhydrous potassium carbonate and 5 ml of neutral dimethyl sulphate were added. The reaction mixture was heated for 11 hours on boiling water bath. Acetone was distilled under reduced pressure and water was added to it. The compound so obtained was filtered and washed well with water and dried under vacuum. The partly methylated compound was further methylated by Purdie's ^{14d} method using methyl iodide and silver oxide. The dried methylated product was taken up in 10 ml methanol. 5 g of silver oxide and 5 g of methyl iodide were added in 5 equal instalments. The reaction mixture was stirred with magnetic stirrer for an hour, after each addition. During the reaction, temperature was kept at 45-55°C. After adding whole methyl iodide and silver oxide, the reaction mixture was refluxed for four hours on a boiling waterbath. The above process was repeated twice for complete methylation. The methylated product was recrystallised from chloroform.

HYDROLYSIS OF THE METHYLATED COMPOUND

The methylated compound was hydrolysed using 4% methanolic hydrogen chloride under reflux for eight hours using calcium chloride guard tube. It was followed by the hydrolysis using aqueous hydrochloric acid on a water bath for 10 hours. The hydrolysate was neutralised with silver carbonate. It was filtered and washed well with hot water and ethanol. The total filtrate was concentrated under reduced pressure to a syrup. The syrup was examined by paper chromatography over Whatman No. 1 filter paper using (a) n-butane : ethanol : water (5:1:4 v/v)¹⁵, (b) methyl ethyl ketone : water (10:1 v/v)¹⁶ as solvents. After the development of chromatogram, papers were air dried and then heated at 120° for 15 minutes. In both the cases a single spot with $R_{\text{TMG}} = 1.00$ (tetramethyl glucose) as reference was obtained. Thus methylated sugar was identified to be 2,3,4,6 tetra-o-methyl D-glucose.

PERIODATE OXIDATION OF COMPOUND C

Compound C (50 mg) was dissolved in 6 ml of pyridine and 15 ml of saturated solution of sodium meta periodate and 90% ethanol was added to it to make it 50 ml in the measuring flask. A blank was also prepared similarly. After 40 hours, aliquots of 5 ml were withdrawn from each of the reaction mixtures.

The periodate consumed and the formic acid produced were estimated by titrimetric method of Jones et al.¹⁷.

Molecular weight of the compound 0 576, for 5 ml aliquots of the solution.

0.011 N sodium thiosulphate (hypo) consumed = 1.88 ml

0.014 N sodium hydroxide consumed = 0.44 ml

For each mole of the compound,

Mole of periodate consumed = 2.04

Mole of formic acid liberated= 1.13

ENZYMATIC HYDROLYSIS OF PHYTOSTEROLIN

Emulsin used for hydrolysis of glycoside was extracted from almonds by the following method¹⁸.

The almond were put in boiling water for one minute and then the brown skin, which has been loosened, was removed. The blanched almonds were crushed in a mortar as thoroughly as possible. 30 ml of water was added to it to make a paste. 10 ml of 10% acetic acid was added to it and it was mixed well and was allowed to stand for 5 minutes. Stirring was done at intervals, then it was filtered through buchner funnel and the residue in the funnel was thoroughly washed with distilled water. One drop of 10% acetic acid was added to the filtrate whereupon the clear solution became turbid, therefore, a little

more of acetic acid was added drop by drop, until no more precipitate was formed. Then it was refiltered. The clear filtrate was used for hydrolysis.

The glycoside (20 mg) was dissolved in aqueous ethanol (20 ml) and to this, emulsin solution (25 ml) was added and the mixture was kept as such for four days. Then the mixture was extracted with amyl alcohol. The aqueous layer was concentrated on rotatory evaporator and the syrup so obtained, on paper chromatographic examination gave a single spot, R_f 0.18 in n-butanol : acetic acid : water (4:1:5 v/v) system spraying with aniline hydrogen phthalate reagent.

IR SPECTRUM

The IR (KBr) of the compound was recorded on Perkin Elmer infraord spectrophotometer. The prominent peaks in the IR spectrum are at 3350, 2890, 1630, 1460, 1380, 1258, 1075, 1020, 810 cm^{-1} .

SECTION - D

CHEMICAL EXAMINATION OF COMPOUND 'D'

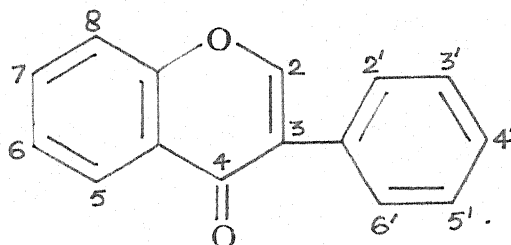
A reddish brown coloured compound D, m.p. 167-9°C was isolated from the stems of Flumbago zeylanica having molecular formula, $C_{22}H_{22}O_{11}$, R_f value 0.92 in n-butanol : acetic acid : water (4:1:5 v/v) system.

The compound D gave positive molisch test, but it neither reduced Fehling's solution nor gave a colour with aniline hydrogen phthalate reagent; thereby indicating that the compound D is a glycoside.

The compound D gave the following colour reactions :

- i) It produced an yellow colour with aqueous sodium hydroxide solution¹⁹
- ii) It produced an yellow colour when treated with a few drops of concentrated sulphuric acid¹⁹.
- iii) It produced an yellow colour when treated with magnesium and hydrochloric acid^{19,20}.
- iv) It gave pink colour when treated with sodium amalgam and hydrochloric acid^{19,20}.
- v) It did not give positive test with 2:4 dinitrophenyl hydrazine²¹ reagent.

From the above observed reactions it is obvious that compound D was an isoflavone derivative and not a flavone and should have the following skeleton :



(vi) It did not respond to positive test with ethanolic boric acid and sodium acetate²²,

(vii) It could not be reduced with sodium borohydride²³,

These two reactions eliminate the possibility of flavone and indicate the presence of isoflavone derivative. Finally the above skeleton of the compound D was further confirmed by the absorption maxima²⁴, at 258 nm and 323 nm.

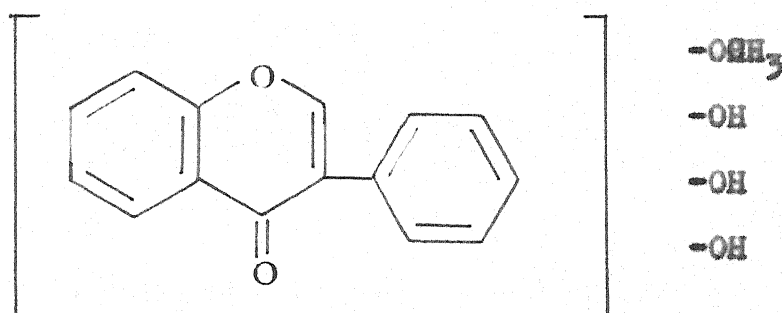
On hydrolysis of glycoside D with 8% methanolic hydrochloric acid gave an aglycone and a sugar moiety.

The sugar was identified as glucose by paper chromatography in n-butanol : acetic acid : water (4:1:5 v/v) system which revealed a single spot with R_f value 0.18. Finally it was confirmed by co-chromatography and formation of osazone derivative m.p. 201°C.

STUDY OF AGLYCON

The aglycone, obtained by the hydrolysis of the glycoside, was yellow coloured solid, $C_{16}H_{12}O_6$, m.p. $230^{\circ}C$. It gave the same colour reactions (i-vii) as described earlier.

The aglycone on treatment with acetic anhydride and pyridine formed triacetyl derivative, $C_{16}H_9O_6(COCH_3)_3$ m.p. $155^{\circ}C$, showing the presence of three free hydroxyl groups. The compound was also found to contain one methoxyl group (Zeisel's method) which was confirmed by IR peak at 2850 cm^{-1} 25(a). Thus the structure of the compound may be represented as follows :

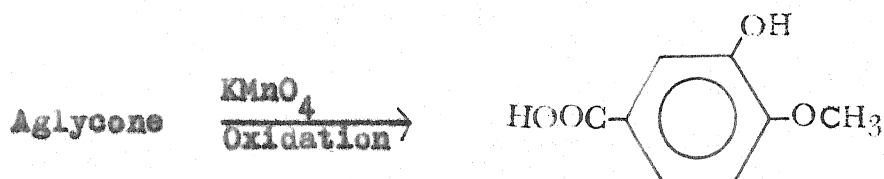


This finally accounted for all the oxygen atoms in the molecule.

The relative positions of methoxyl and of the hydroxyls have been determined by the degradation studies, colour reactions and absorption spectra of the aglycone.

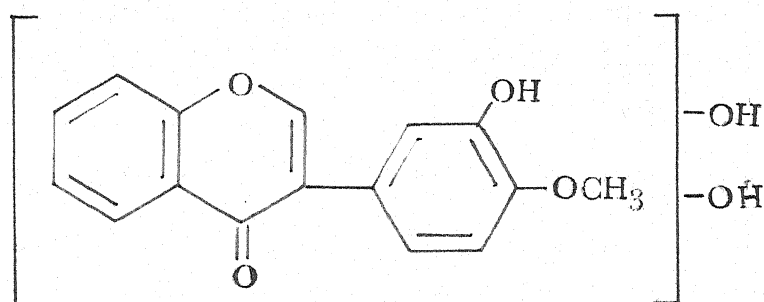
When the aglycone was oxidised with neutral potassium permanganate, ortho-hydroxy-anisic acid

(isovanillic acid) was one of the products, isolated from the oxidation mixture.



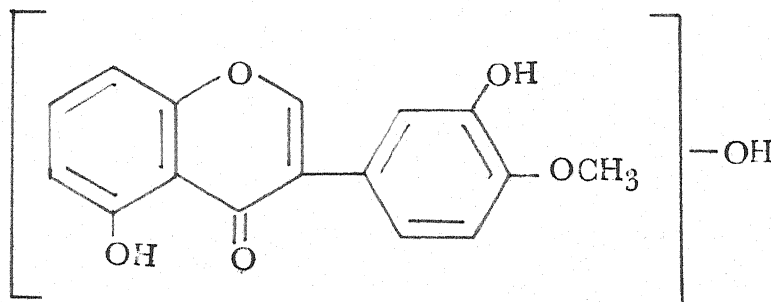
This clearly places one hydroxyl and a methoxyl groups at position 3' and 4' respectively.

Thus the structure may be further written as follows:



The aglycone gave a green colour with ethanolic ferric chloride²⁶ and a positive reaction with ethanolic boric acid in presence of citric acid²⁷, showing the presence of a free hydroxyl at position-5. Finally it was confirmed by the fact that isoflavone having a free 5-hydroxyl group form complex with aluminium chloride²⁸ and that this complex formation results a bathochromic shift^{29,30} of 13 nm in the spectra of the compound on the addition of 1% ethanolic solution of aluminium chloride. It was further supported by the carbonyl absorption at 1660 cm^{-1} shown by the compound revealed the presence of chelated hydroxyl at 50-position.

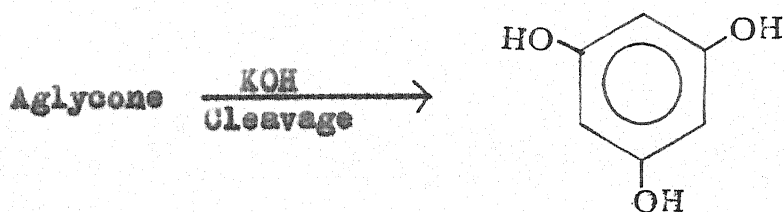
Now the structure of the aglycone may be mentioned as given below :



The aglycone gave positive test with fused sodium acetate and with vanilline hydrochloric acid reagent³⁴, showing that remaining hydroxyl group is present at position-7.

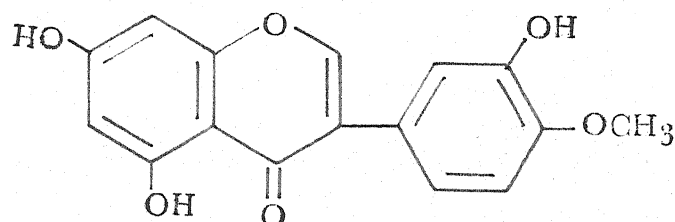
The presence of free hydroxyl group at position-7 has been confirmed by the following two facts :

- A bathochromic shift of 11 nm (λ_{max} changed from 324 nm to 335 nm) was observed on addition of fused sodium acetate solution to the ethanolic solution of the compound³².
- The cleavage of aglycone with 5% aqueous potassium hydroxide gave a phloroglucinol molecule.



Phloroglucinol

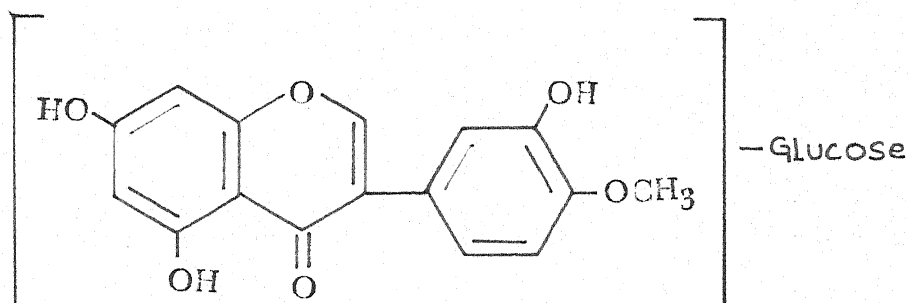
Thus the structure of the aglycone was as follows :



(5,7,3'-trihydroxy-4'-methoxy isoflavone)

STUDY OF THE GLYCOSIDE

On the basis of the above facts the structure of compound D may be represented as :



Now the nature and position of the linkage of sugar moiety have to be decided from its structure. It is obvious that it may be either 3' or 5 or 7, another point to be decided, is whether the sugar linked in the α or the β form.

The position of the sugar linkage has been ascertained as follows :

- a) Both the glycoside and the aglycone exhibited a bathochromic shift of 16-19 nm with sodium methoxide showing the presence of free hydroxyl group in position 3'.
- b) Both the glycoside and the aglycone showed a bathochromic shift of 13-18 nm with ethanolic aluminium chloride showing the presence of free hydroxyl in position-5. This conclusion was also supported by the green colour produced when both are treated separately with ethanolic ferric chloride. They also gave positive reaction with ethanolic boric acid in presence of citric acid.

It is evident from the above discussion that in the glycoside the position 3' and 5 have free hydroxyl groups and so the only hydroxyl group of the aglycone left for glycosidation was at C-7 which is further confirmed by the following observations.

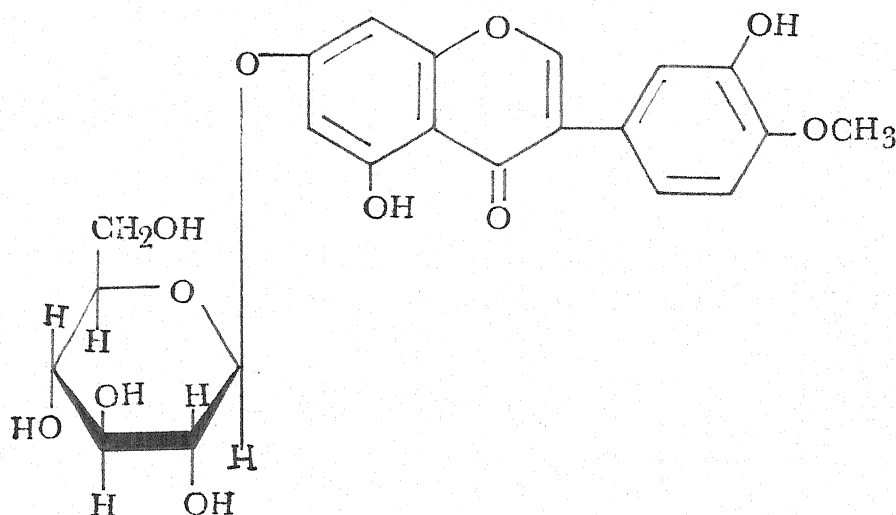
- a) No bathochromic shift was observed when sodium acetate was added to the ethanolic solution of the glycoside showing that the hydroxyl group at position-7 was substituted³².
- b) The glycoside did not give colour with Vanillin and hydrochloric acid.

From the above facts it is confirmed that the glucose is attached with aglycone at position-7. The glycosidic nature of the compound^{25b} was reflected by strong absorption bands at 1060 and 1120 cm^{-1} .

On periodate oxidation the compound D consumed 2.1 moles of periodate and liberated 1.1 mole of formic acid per mole of the glycoside, indicating the presence of only one unit of glucose present in the pyranose form.

The compound D got hydrolysed with emulsin which is specific for β -linkage. Thus the sugar is linked with the aglycone through β -linkage.

Thust most probable structure of the compound D has been represented finally as 3',5-dihydroxy-4'-methoxy isoflavone 7- β -glucopyranoside.



COMPOUND - D

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound D was obtained from ethanolic extract of the stems of Plumbago zeylanica as described on page 23. The compound D so obtained was purified by repeated crystallisation from ethyl acetate : methanol (1:1). It gave a red brown coloured compound D, m.p. 167-9°C having molecular formula $C_{22}H_{22}O_{11}$. The purity of the compound was tested on paper and thin layer chromatography.

CHROMATOGRAPHY

Descending technique of paper chromatography was done using Whatmann No. 1 filter paper and following three solvent systems were taken,

- a) n-Butanol : acetic acid : water (4:1:5 v/v) system.
- b) Acetic acid : concentrated hydrochloric acid : water (30:3:10 v/v) system.
- c) n-Cresol : acetic acid : water (50:2:48 v/v) system.

R_f found 0.92 in solvent system (a) 0.86, in (b) and 0.69 in solvent system (c) respectively.

The spots were developed by exposing the paper in the vapours of ammonia.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{22}H_{11}O_{11}$</u>
C = 57.10%	C = 57.14%
H = 4.81%	H = 4.76%
Mol.wt. = 462	Mol.wt. = 462
(Rast's method)	(Calculated)

HYDROLYSIS OF GLYCOSIDE

The compound D (200 mg) was hydrolysed with 8% methanolic hydrochloric acid for 10 hours on a boiling water bath. After cooling the contents were extracted with ether. The aqueous portion was neutralised with silver carbonate and filtered. The filtrate was concentrated to a syrup.

IDENTIFICATION OF SUGAR

The syrup was identified by paper chromatography as given on page 51 . It gave R_f value 0.18 in n-butanol : acetic acid : water (4:1:5 v/v) system. On treatment with phenyl hydrazine reagent, it formed an osazone m.p. $204^{\circ}C$ suggesting the sugar to be glucose.

IDENTIFICATION OF AGLYCONE

The ether layer was washed well and the solvent was evaporated. The solid mass was recrystallised from ethyl acetate as a light yellow compound m.p. $230^{\circ}C$. The

purity of the compound was tested on thin layer chromatography, using the following solvent systems :

- i) Ethyl acetate saturated with water.
- ii) Ethyl acetate : benzene (75:25 v/v)
- iii) Ethyl acetate : formic acid : water (70:15:15 v/v)
- iv) n-Butanol : formic acid : water (85:5:10 v/v)

The single spot was observed when the plates were exposed to the vapours of ammonia.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{16}H_{12}O_6$</u>
C = 63.6%	C = 64.0%
H = 4.4%	H = 4.0%

ACETYLATION OF THE AGLYCONE

Aglycone (30 mg) was taken with 4 ml of acetic anhydride and 2 ml of pyridine. After keeping the solution overnight, it was poured in crushed ice with continuous shaking. The precipitate so formed was filtered and crystallised from acetone, m.p. 155°C.

DETERMINATION OF ACETYL GROUP PERCENTAGE

The percentage of acetyl group in the acetylated product was determined by the method of Wiesenberger³³ as described by Belcher and Godbert³⁴.

<u>Found</u>	<u>Calculated for $C_{16}H_{19}O_6(COCH_3)_3$</u>
Percentage of acetyl group = 28.26	Percentage of acetyl group = 30.28

POTASSIUM PERMANGANATE OXIDATION

The aglycone (30 mg) of compound D was treated with neutral potassium permanganate under reflux for four hours. The reaction mixture was cooled and excess of manganese dioxide removed by adding sodium bisulphite to it. On addition of hydrochloric acid, a solid compound was obtained, filtered out and crystallised from ethanol. It was found to melt at 253°C which was identified as ortho-hydroxy anisic acid (lit. m.p. 255°C). It was confirmed by co-chromatography and mixed m.p. with authentic sample.

CLEAVAGE OF THE AGLYCONE OF COMPOUND D WITH 5% AQUEOUS POTASSIUM HYDROXIDE

The aglycone (30 mg) was treated with 25 ml of 5% aqueous potassium hydroxide. According to the method of Harborne and Hirst³⁵, the reaction mixture was refluxed for two hours. The contents were cooled and excess of alkali destroyed by adding dilute hydrochloric acid. An extra 5 ml of distilled water was added to the reaction mixture and whole mixture was shaken with ether. The etherial layer was separated and treated with anhydrous sodium sulphate.

On concentration of ether solution a substance was obtained. It was recrystallised from ethanol and was identified as 2:4:6 trihydroxy anisole by mixed m.p. and co-chromatography with an authentic sample.

DETERMINATION OF METHOXYL GROUP (ZEISEL'S METHOD)

The determination of methoxyl group in aglycone of compound D was carried out according to the well known Zeisel's method modified by Belcher, Fildes and Nutten³⁶.

The spiral scrubbing compartment of the apparatus was filled with 3.5-4.0 ml of antimony potassium tartrate solution (10%) in water and the stopper replaced. 10 ml of freshly prepared bromine-sodium acetate solution in acetic acid (prepared by adding 0.3 ml of bromine to 10 ml of a 10% solution of sodium acetate in acetic acid) were added in the receiver which was connected to the scrubbing compartment.

The aglycone (20 mg) accurately weighed in a small glass-cup was allowed to slide down gently into the boiling flask. A drop of mercury was introduced followed by the addition of 5 ml of melted phenol (AR) and 5-6 drops of propionic anhydride were also added. The compound was dissolved in this solution mixture before the addition of 5 ml of hydroiodic acid. The connections were made gas tight. Carbon dioxide gas was passed through the apparatus at the rate of 60-70 bubbles per minute. The contents were

heated and slowly brought to boil and the temperature adjusted so that the vapours did not rise more than half way to the condenser.

After heating for three hours the receiver and spiral inlet tube were removed from the washing compartment and rinsed with distilled water. The contents of the receiver and washing were collected into a 250 ml Erlenmeyer flask containing 5 ml of 10% sodium acetate solution.

Formic acid was added dropwise to the solution till the excess of bromine was destroyed, then 0.5 gm of potassium iodide and 5 ml of 10% sulphuric acid were added. The contents were treated after five minutes with standard 0.05N sodium thiosulphate solution using starch as indicator. A blank was also carried out simultaneously.

The percentage of methoxyl group was calculated by the amount of hypo consumed for titrated methyl iodide produced for each mole of the compound.

<u>Found</u>	<u>Calculated for $C_{15}H_{13}O_5(OCH_3)$</u>
Percentage of methoxyl group = 11.36	Percentage of methoxyl group = 10.33

PERIODATE OXIDATION

The compound D (25 mg) was dissolved in 20 ml aldehyde free ethanol (95%) and to it saturated sodium

meta periodate solution (20 ml) in same ethanol was added and made it up to 50 ml in a measuring flask. A blank was similarly prepared in another 50 ml measuring flask. After 40 hours, aliquots of 5 ml were withdrawn from both the reaction mixtures. The periodate consumed was estimated by titration against standard hypo solution and the formic acid liberated by titrating against standardised sodium hydroxide solution according to the method of Jones et al.³⁷

Molecular weight of compound D = 462.00

For 5 ml of the solution

0.011 N sodium thiosulphate (hypo) consumed = 2.3 ml

and 0.011 N sodium hydroxide consumed = 0.58 ml

For each mole of the compound,

Mole of periodate consumed = 2.1

Mole of formic acid liberated = 1.1

ENZYMATIC HYDROLYSIS OF GLYCOSIDE

The emulsin (described on page 54) was used for its enzymatic hydrolysis which is specific for β -linkage. The glycoside got completely hydrolysed and the sugar so obtained, was chromatographed on Whatmann No. 1 filter paper.

ABSORPTION SPECTRA

The UV spectra of the compound D was taken on Perkin Elmer 202 spectrometer.

Solution and Reagent	λ_{max} (nm)	Shift (nm)
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SPECTRA OF GLUCOSIDE

a) Ethanolic solution	258, 323	-
b) Ethanolic solution + AlCl_3	341	18
c) Ethanolic solution + NaOCH_3	342	19

SPECTRA OF AGLYCONE

a) Ethanolic solution	260, 324	-
b) Ethanolic solution + AlCl_3	337	13
c) Ethanolic solution + NaOAc	335	11
d) Ethanolic solution + NaOMe	340	16

IR SPECTRUM

A Perkin-Elmer Infracord spectrophotometer was used for the IR spectras (KBr).

a) The prominent peaks of the compound D in IR spectrum are at 3440, 2940, 2850, 1760, 1660, 1610, 1230, 1120, 1060, 840, 820, 760 cm^{-1} .

b) The prominent peaks of the aglycone in IR spectrum at 3445, 2930, 2850, 1760, 1665, 1525, 1220, 1120 cm^{-1} .

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CHAPTER - III

CHEMICAL EXAMINATION OF TINOSPORA CORDIFOLIA

The plant Tinospora cordifolia, commonly known as Guranch, belongs to the family 'Menispermaceae'. It is found throughout tropical India.

The plant of Tinospora cordifolia is a large twiner; stem succulent; bark light grey, papery at first and later corky. Leaves cordate, long stalked. Flowers on nodes on the old wood, in racemes, dioecious, very small, yellow. Drupes sessile, red, very small.

It is known to be of great medicinal importance¹. The fresh plant is considered more efficacious than the dried plant. It is antiperiodic, alterative tonic, hepatic stimulant and diuretic. The aqueous extract of the plant called Indian quinine, is very effective in ordinary fevers due to cold and indigestion; the plant is commonly used in rheumatism, urinary diseases, dyspepsia, general debility, syphilis, skin diseases, biliousness, piles, bronchitis, impotence, jaundice and torpidity of liver.

The fecula prepared from the roots and stems is highly valued drug for intermittent fevers, chronic diarrhoea, some forms of chronic dysentery, secondary syphilis and leucorrhoea. A necklace of stems or roots is usually worn as a cure of jaundice. The paste of the plant is plastered over a fractured bone.

The work done in the past years on this plant was surveyed and the details of it are given below :

Constituents	Parts of Plant	References
1. Glycerides of myristic and Palmitic acids, Octacosanol, and two bitter principles.	Stems	Bhide, B.V., Phalnikar, N.L. and Paranjpe, K. ²
2. Giloin, Giloinin, Gilonisterol	Stems	Kidwai, A.R., Salooja, K.C., Shamma, V. and Siddiqui, S. ³
3. An alkaloid, β -sitosterol, a fatty acid	Stems	Seghal, S.N. and Majumdar, D.N. ⁴
4. 2 Bitter principles (tinosporide and cordifolide), an alkaloid-tinosporin and δ -sitosterol	Plants (stems and leaves)	Khuda, M.Q., Khaleque, A. and Roy, N. ⁵
5. Tinosporon, Tinosporic acid, Tinosporol	Stems	Khuda, M.Q., Khaleque A., Bashir, K.A., Khan, M.A.R. and Roy, N. ⁶
6. Heptacosanol, cordifol, β -sitosterol, tinosporidine and cordifolone	Stems and leaves	Khaleque, A., Miah, M.A.W., Huq, M.S. and Khan, A.B.K. ⁷
7. Tinosporidine, β -sitosterol, cordifol heptacosanol.	Stems (fresh)	Ibid ⁸
8. Octacosanol, β -sitosterol and unidentified compound	Stems	Dixit, J.N. and Khosa, R.L. ⁹

From the survey of literature it seems that no work has been done on the roots of the plant. Therefore, it was considered worthwhile to study the active chemical constituents from the roots of the plant which make the contents of the present chapter.

EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS OF ROOTS OF TINOSPORA CORDIFOLIA

The roots of Tinospora cordifolia were collected locally and identified for their authenticity in the Botany Department of D.V. College, Orai.

Dried and crushed roots (2.0 kg) were defatted with petroleum ether (60-80°C) in a soxhlet extractor for 20 hours. The extract was concentrated into small volume. The excess of methanol was added in this concentrated extract. A white solid mass was precipitated which was filtered off over a sintered glass crucible. The filtrate was concentrated and chromatographed over silica gel G. A white crystalline compound (E) was obtained with petroleum ether : ether (1:2). The white solid mass to the other hand was washed well and crystallised from chloroform to give compound (F).

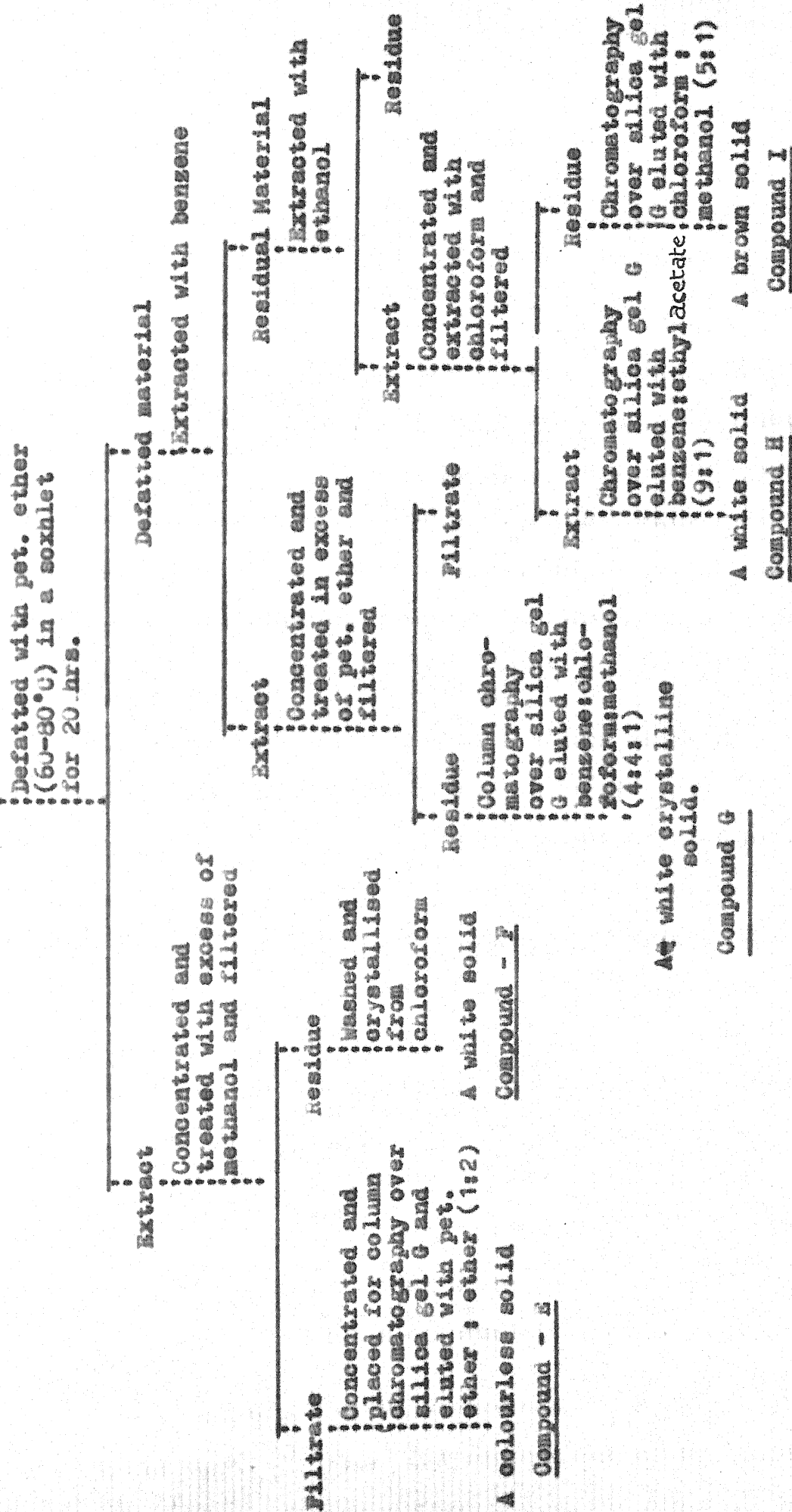
The defatted material was extracted with benzene several times. The extracts were combined and concentrated

to a very small volume. Later on the roots were extracted with ethanol (95%) in several lots. The total extract was concentrated at reduced pressure to a brown viscous mass.

The concentrated benzene extract was treated with excess of petroleum ether (40-60°C) to give a dirty white precipitate. It was filtered off, dried and placed for column chromatography over silica gel G. The solvent system, benzene : chloroform : methanol (4:4:1 v/v) gave a white crystalline compound G.

The brown viscous mass was refluxed with chloroform on a water bath and filtered. The filtrate was concentrated and placed over silica gel G for chromatography. Benzene : ethyl acetate (1:9) solvent system gave a white crystalline solid (H). The residual mass was placed for column chromatography over silica gel G. A yellow brown compound (I) was obtained with chloroform : methanol (5:1 v/v).

STEMS OF TIMOSPOHA CORDIFOLIA



SECTION - A

CHEMICAL STUDY OF COMPOUND E

The compound E, m.p. 135-37°C, $[\alpha]_D^{31} -35.5^\circ$, was isolated from the roots of Tinospora cordifolia as described on page 77. The elemental analysis of the compound gave molecular formula $C_{29}H_{50}O$, also in agreement with molecular ion peak at m/e 414 in its mass spectrum. It was soluble in petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol.

The compound E gave positive colour tests in Liebermann-Burchard reaction¹⁰, Salkowski reaction¹¹, Tsugajew reaction¹² and Kohlenberg's reaction¹³. It also gave red colour with Moller's reagent¹⁴. These colour reactions are specific for steroids and triterpenoids. Since the compound did not produces a violet colour in the Brieskorne test¹⁵, it does not belong to triterpenoids. From the molecular formula and colour reactions, it is evident that the compound E is a sterol. It also gave positive test with tetranitromethane¹⁶, indicating the presence of an olefinic bond in the molecule, which is further supported by the peaks at 842 cm^{-1} and 845 cm^{-1} in the IR spectrum¹⁷ of the compound (Fig. III.1).

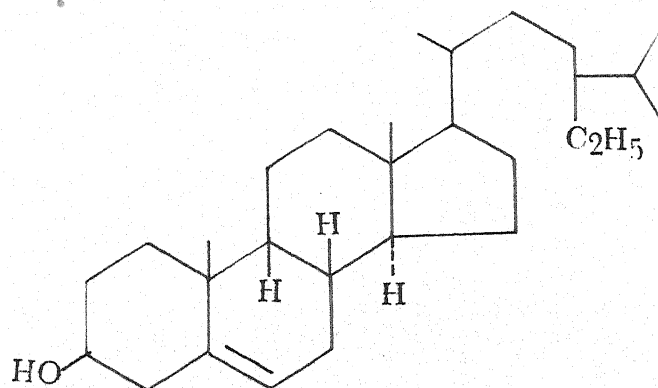
The compound gave a monoacetate, $C_{31}H_{52}O_2$,
m.p. 128-29°C, $[\alpha]_D^{31} -35.9^\circ$ (in benzene) on acetylation

and a monobenzoate, $C_{36}H_{54}O_2$, m.p. $142^\circ C$, $[\alpha]_D^{28} - 14.2^\circ$ (in chloroform) on benzylation. It indicates the presence of hydroxyl group in the molecule. The IR spectra of the compound gave a peak at 3490 cm^{-1} ^{17,18}, characteristic of hydroxyl group, which is further confirmed by the hydroxyl group proton signal in NMR spectra centered at $\delta 5.35$ (triplet).

The NMR spectrum of the compound reveals the presence of five methyl groups centered at $\delta 0.68$ (s) for methyl group C-13, $\delta 0.88$ (+) for methyl group at C-28, $\delta 0.93$ (d) for two methyl groups at C-25, $\delta 0.96$ (d) for methyl group at C-20 and $\delta 1.00$ (s) for methyl group at C-10.

From the above studies it was found that properties of the compound E and its derivatives correspond to those of β -sitosterol and its derivatives. It was confirmed by mixed m.p., co-chromatography and superimposition of its infra-red spectra with authentic sample of β -sitosterol ¹⁸.

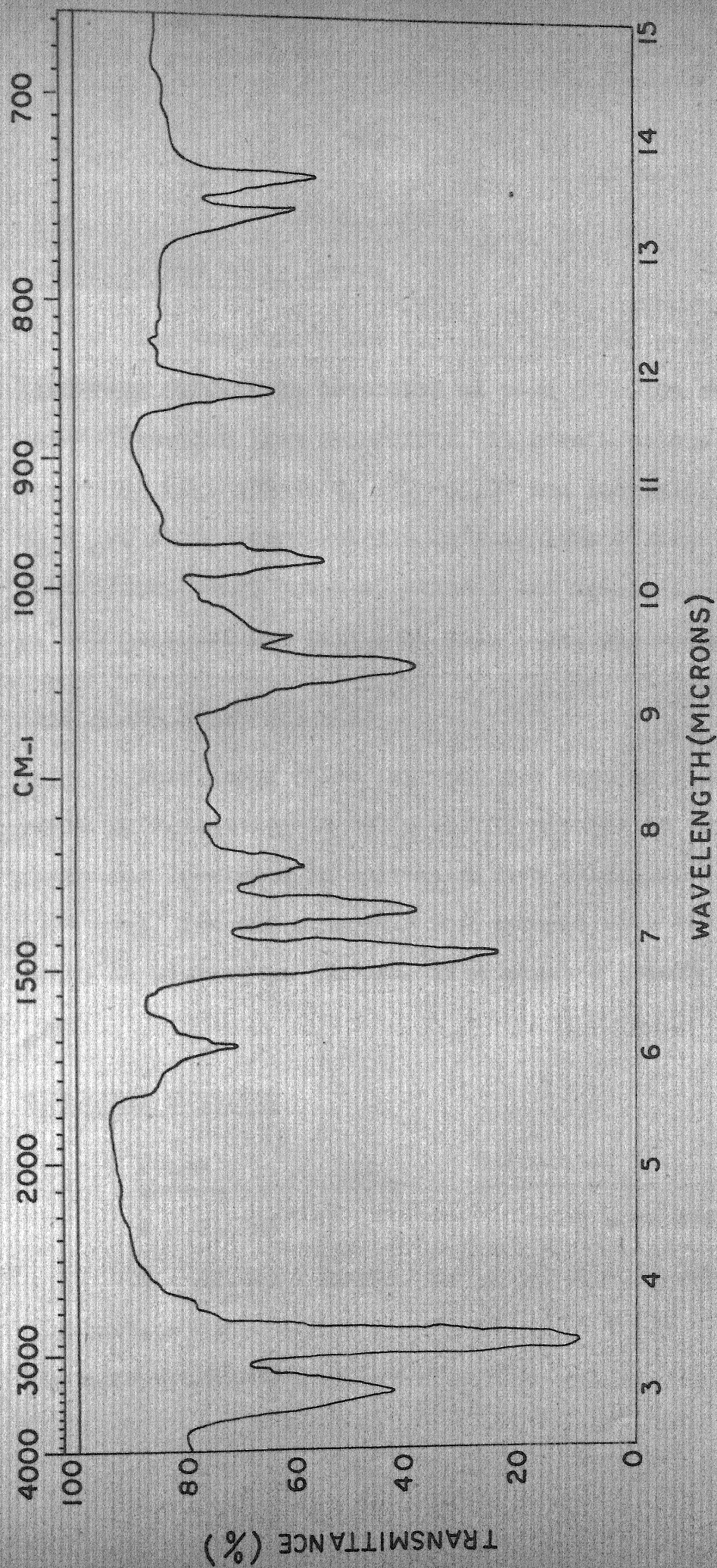
The structure of the compound E is given below :



(Compound E)

This structure was further confirmed by mass spectrum of the compound, giving the prominent ion peaks at m/e 414 (M^+), 400, 382, 362, 273, 231 and 199.

FIG. III. I. INFRA RED (KBr) SPECTRUM OF COMPOUND - E



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound E was isolated from the roots of Tinospora cordifolia described on page 77 . On repeated crystallisation from chloroform, it gave a colourless compound, m.p. 135-37°C, $[\alpha]_D^{31} -35.5^\circ$ and molecular formula $C_{29}H_{50}O$. It was very soluble in petroleum ether, benzene, chloroform, ethyl acetate, ethanol and methanol. The purity of the compound was tested on thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plate by activating at 100°C for 15 minutes in an oven. The plate was developed in solvent system chloroform : benzene (1:1 v/v). The chromatoplate was sprayed with 2N- H_2SO_4 , which on heating to 110° for five minutes gave a single spot.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{29}H_{50}O$</u>
C = 84.18%	C = 84.06%
H = 12.14%	H = 12.08%
Mol. wt. = 414	Mol. wt. = 414
(By Mass spectrum)	

COLOUR REACTIONS

The compound gave following colour reactions :

- i) Deep red colour in Liebermann-Burchard reaction.
- ii) Yellow colour changing to red in Salkowskii reaction.
- iii) Red colour with a greenish yellow fluorescence in Tsugajew reaction.
- iv) Deep purple colour in Kohlenberg's reaction.
- v) Red colour with Noller's reagent.

ACETYLATION OF THE COMPOUND E

The compound (20 mg) was acetylated as usual as described on page 50 . The acetylated product was crystallised from methanol as white flakes, m.p. 128-29°, $[\alpha]_D^{31} -35.9^\circ$.

ELEMENTAL ANALYSIS OF ACETYL DERIVATIVE

<u>Found</u>	<u>Calculated for $C_{31}H_{52}O_2$</u>
C = 81.35%	C = 81.57%
H = 11.45%	H = 11.40%

Acetyl percentage found = 10.26% (Wiesenberger's¹⁹ method)
Calculated = 9.42%

BENZOYLATION OF THE COMPOUND E

The compound (30 mg) was benzoylated as usual with benzoyl chloride and pyridine as described on page 33 . The benzoylated product was crystallised from methanol, m.p. 142°C, $[\alpha]_D^{28} -14.2$.

DIGITINOIDE DERIVATIVE OF COMPOUND E

Compound (50 mg) in 5 ml hot ethanol was mixed with 5 ml of saturated solution of digitonin in absolute ethanol and the mixture was heated on water bath for 20 minutes and then cooled. A precipitate so obtained was separated, filtered, dried and crystallised from ethanol as a flocculant white solid, m.p. 216-18°C (lit. m.p. 213°).

CO-CHROMATOGRAPHY OF COMPOUND E

Thin layer chromatography of the compound along with an authentic sample of β -sitosterol was carried out on silica gel G plate using chloroform : benzene (2:1 v/v) as developing solvent. The spots were detected by spraying the plate with 2N H_2SO_4 and heating at 100°C. The R_f value of the spot was found to be at 0.58.

INFRA RED SPECTRUM OF THE COMPOUND E

The compound E gave following main peaks in the IR spectrum (KBr) which was recorded on Perkin-Elmer infrared spectrophotometer.

3490 (sh), 2950 (s), 2855 (w, shoulder), 1640 (broad), 1470 (s), 1390 (s), 1268 (sh), 1198 (w), 1136 (w), 1060 (s), 1026 (w), 965 (m), 845 (w), 842 (w), 806 (m), 765 (s) and 740 (s) cm^{-1} .

NMR SPECTRUM OF THE COMPOUND E

The NMR of the compound E was taken on Varian A-60D spectrometer, CDCl_3 as solvent and TMS as reference.

<u>Signals in δ value</u>	<u>Assignments</u>
0.68 (s)	methyl group at C-13
0.88 (t) ($J = 6$ cps)	methyl group at C-28
0.93 (d) ($J = 2.40$ cps)	2 methyl group at C-25
0.96 (d)	methyl group at C-20
1.00 (s)	methyl group at C-10
1.08-2.40 (m)	methylenic groups
5.35 (t)	ethylenic hydroxyl group

MASS SPECTRUM OF THE COMPOUND E

The compound gave following prominent ion peaks in its mass spectrum on Hitachi RMU-6-E spectrometer, m/e 414 (M^+), 400, 382, 362, 273, 231 and 199.

SECTION - B

STRUCTURAL STUDY OF THE COMPOUND F

A white compound F, m.p. 80-83°C, was isolated from the roots of Tinospora cordifolia as described on page 77. The elemental analysis of the compound gave molecular formula $C_{28}H_{58}O$. It gave pink colour with ceric ammonium nitrate showing the presence of alcoholic group in the compound.

The compound F did not decolourise potassium permanganate solution or bromine in carbon tetrachloride, indicating it to be a saturated compound. Further it did not show any absorption in ultraviolet region showing the absence of any chromophoric group. It has been found to be aliphatic in nature as revealed by the absorption bands at 2857, 2788, 1468, 732 and 720 cm^{-1} in its infra red spectrum^{20,21,22}.

The infra red spectrum of the compound F shows stretching due to polymeric association of hydroxyl group involving in inter molecular hydrogen bonding at 3226 cm^{-1} ²³. A peak obtained at 1340 cm^{-1} ²⁴ due to -OH bending vibration of primary alcohols and a peak at 1060 cm^{-1} due to stretching vibration of primary alcohols²⁵, clearly show that the molecule is a primary alcohol²⁶. A peak obtained at 2857 cm^{-1} is for C-H stretching and a peak obtained at 1468 cm^{-1} is due to -CH₂ bending vibrations^{27,28}. A doublet at 732 cm^{-1} and

722 cm^{-1} was indicated that compound has a long chain. This doublet is a characteristic of the compound containing more than four $-\text{CH}_2$ groups in a long straight chain²⁹. The above facts suggested that the compound is a long chain saturated aliphatic primary alcohol.

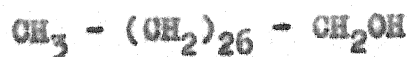
On acetylation, the compound F formed a monoacetate $\text{C}_{28}\text{H}_{57}\text{OOCOCH}_3$, m.p. 64°C . Further on oxidation with concentrated nitric acid, the compound yielded a monocarboxylic acid m.p. 98°C . The elemental analysis of the acid gave molecular formula $\text{C}_{28}\text{H}_{56}\text{O}_2$ corresponding to n-octacosanoic acid. The IR spectrum of the acid shows a peak at 1725 cm^{-1} due to carboxylic group whereas no peak in the hydroxyl region. It also confirmed the primary nature of the hydroxyl group.

The NMR spectrum^{21,30,31} of the compound also confirmed it to be a long chain primary alcohol. It showed a triplet at $\delta 0.85$ due to terminal methyl group. A strong unresolved band at $\delta 1.22$ was for protons of $(\text{CH}_2)_n$ group and two protons of C-1 methylene group (adjacent to hydroxyl group) appeared as a triplet at $\delta 3.60$. A singlet was obtained for the hydrogen of hydroxyl group at $\delta 1.72$.

The mass spectrum of the compound shows a prominent peak at m/e 392 ($\text{M}^+ - \text{H}_2\text{O}$) which is supported by the fact that in the case of long chain alcohols molecular ion peak is not observed due to the thermal decomposition but a peak at m/e ($\text{M}^+ - \text{H}_2\text{O}$) is observed^{21,32}. Elimination of water is followed

by the expulsion of ethylene leading to a peak at m/e 364 ($M^+ - H_2O - CH_2 = CH_2$). It also contains a set of alkane type (m/e 29, 43, 57.....) and olefinic type (m/e 41, 42, 55, 56.....) fragments. At high mass end of spectrum ions occurred at the interval of fourteen mass unit as expected for a long chain primary alcohols^{33,34}.

On the basis of the above facts and the formation of *n*-octacosanoic acid as the oxidation product and octacosanyl acetate as acetylated product, led to the confirmation of compound *P* as a straight chain aliphatic alcohol, *n*-octacosanol.



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound F was isolated from the roots of Tinospora cordifolia as described on page 77 from the petroleum ether extract by precipitating with methanol. On repeated crystallisation from chloroform : methanol (1:1) mixture, gave a white compound, m.p. 80-83°C, molecular formula $C_{36}H_{64}O_6$. The compound was soluble in petroleum ether, benzene, chloroform and ethanol but insoluble in acetone and methanol. The purity of the compound was tested by thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plates by activating the plates 120°C for 30 minutes in an electric oven. Using solvent system n-hexane : ethyl acetate (75:25 v/v). A single spot was observed on spraying with 50% sulphuric acid followed by heating at 120°C for 15 minutes. It have R_f value 0.68.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{28}H_{58}O$</u>
C = 81.79%	C = 81.95%
H = 14.11%	H = 14.15%
Mol. wt. = 410	Mol. wt. = 410
(By Mass Spectrometry)	

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound F was isolated from the roots of Tinospora cordifolia as described on page 77 from the petroleum ether extract by precipitating with methanol. On repeated crystallisation from chloroform : methanol (1:1) mixture, gave a white compound, m.p. 80-83°C, molecular formula $C_{36}H_{64}O_6$. The compound was soluble in petroleum ether, benzene, chloroform and ethanol but insoluble in acetone and methanol. The purity of the compound was tested by thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plates by activating the plates 120°C for 30 minutes in an electric oven. Using solvent system n-hexane : ethyl acetate (75:25 v/v). A single spot was observed on spraying with 50% sulphuric acid followed by heating at 120°C for 15 minutes. It have R_f value 0.68.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{28}H_{58}O$</u>
C = 81.79%	C = 81.95%
H = 14.11%	H = 14.15%
Mol. wt. = 410	Mol. wt. = 410
(By Mass Spectrometry)	

CHARACTERISTIC REACTIONS

- a) It was aliphatic in nature.
- b) Its solution gave pink colour with ceric ammonium nitrate.
- c) It did not decolourise bromine solution or potassium permanganate solution.
- d) No colour with 2:4 dinitrophenyl hydrazine.

ACETYLATION OF THE COMPOUND

The compound (50 mg) were taken in a round bottom flask. To this 15 ml acetic anhydride and 5 ml pyridine were added. The reaction mixture was refluxed for 3 hours in an oil bath between 110 to 130°C. The mixture was cooled and poured into ice cold water. A white mass was precipitated which was filtered, washed, dried and crystallised from chloroform. It was found to melt at 64°C.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{28}H_{57}O_2$</u>
C = 79.20%	C = 79.64%
H = 13.15%	H = 13.27%

DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetyl derivative was quantitatively determined by the method of Wisenberger¹⁹ as described by Belcher and Godbert.

Found = 9.45

Calculated for $C_{28}H_{57}O_2$ = 9.59

The percentage of acetyl group corresponds to one acetyl group. This shows that there was only one hydroxyl group in the compound.

OXIDATION OF THE COMPOUND

The compound (100 mg) and 3 ml of concentrated nitric acid were taken in a 25 ml conical flask fitted with air condenser. The contents of the flask were heated on a sand bath at 120-130° for 6 hours, cooled the contents, poured into ice cold water and left for overnight.

The solution was then extracted with ether. The ethereal layer was separated and washed with distilled water to remove mineral acid. On distilling off ether, a solid mass was obtained which was crystallised from acetone to give an acid, m.p. 90°C. It gave effervescences with aqueous sodium bicarbonate. The IR peak spectrum of the acid showed a peak at 1725 cm^{-1} due to carboxylic group whereas no peak is observed in the hydroxyl region.

ELEMENTAL ANALYSIS OF THE ACID

<u>Found</u>	<u>Calculated for $C_{28}H_{56}O_2$</u>
C = 79.35%	C = 79.25%
H = 13.10%	H = 13.20%

EXAMINATION OF ACID BY PAPER CHROMATOGRAPHY^{35,36}

The paper chromatography of the oxidized product was carried out on Whatman No. 1 filter paper impregnated with 10% solution of liquid paraffin in benzene. Acetic acid : water (7:1 v/v) was used as a developing solvent. The developed chromatogram was heated at 80-100° and was then immersed in copper acetate solution (500 ml of water containing 10 ml of saturated solution of copper acetate). The paper was washed with 0.01% acid was then dipped into a solution of 1.5% aqueous $K_4Fe(CN)_6$. A single brown spot having R_f 0.61 was observed indicating clearly the presence of a single compound.

INFRA RED SPECTRUM OF THE COMPOUND

The IR spectrum (KBr) of the compound was recorded on Perkin-Elmer Infra Cord Spectrophotometer which gave following main peaks.

<u>Position of absorption cm^{-1}</u>	<u>Assignment</u>
3226 (sh)	O-H stretching vibration ³⁷
2857 (s)	C-H stretching in $-CH_2$ ^{30b}
2788 (s) shoulder	
1468 (s)	C-H bending in CH_2 ^{30b}
1380 (w)	C- CH_3 bending ²²
1340	-OH bending vibration ²⁴
1060 (b)	C-O stretching ³⁷ of a saturated primary alcohol.
732 (s)	$(CH_2)_n$ $n \geq 4$ bending due to ^{20,22} straight chain methylene group.
720 (s) doublet	

NMR SPECTRUM OF THE COMPOUND F 21,31

A Varian A-60D spectrometer was used to record the NMR spectra of the compound, CDCl_3 as solvent and TMS as reference.

<u>Signal in δ value</u>	<u>Assignments</u>
0.85	terminal methyl group
1.22	methylene group
1.72	C_1 - methylene group
3.62	proton on hydroxyl group.

MASS SPECTRUM OF THE COMPOUND

The compound gave the following main peaks in its mass spectrum which was recorded on Hitachi RMU-6E spectrometer.

Peaks at m/e 392, 378, 364, 360, 336, 322, 308, 293, 279, 265, 251, 237, 223, 209, 195, 181, 167, 153, 139, 125, 111, 97, 83, 69, 57, 56, 43, 42, 41, 29 and 28.

SECTION - C

STRUCTURAL STUDY OF COMPOUND G

The compound G, m.p. 183-5°C was isolated from the roots of Tinospora cordifolia as described on page 77. The elemental analysis of the compound gave molecular formula $C_{21}H_{30}O_2$ which was also in agreement with molecular ion peak at m/e 314 in its mass spectrum (Fig. III.2). It was soluble in pyridine, hot ethanol and hot methanol.

The compound gave positive colour test of Liebermann Burchard reaction¹⁰, Salkowski reaction¹¹ and Tschugajew reaction¹². It also gave orange colour with Noller's reagent¹⁴. The above colour reactions are specific for steroids and triterpenoids. The compound did not produce a violet colour in Brieskrone test¹⁵, indicating the presence of steroids. From the molecular formula, colour reactions, it is evident that compound G is a sterol. Further selenium distillation of it at 360°, gave Diel's hydrocarbon, m.p. 130°C, which indicated the presence of steroid nucleus.

It decolourised the colour of bromine in carbon tetrachloride and potassium permanganate solution showing the unsaturation in the molecule. Its UV spectrum also shows a absorption maxima at 222 nm³⁸. On bromination it formed a derivative, $C_{21}H_{30}O_2Br_4$, m.p. 226-8°C, indicating the presence

of two olefinic bonds. Further it gave white crystalline precipitate with saturated solution of maleic anhydride which suggested that both double bonds were conjugated.

It is further supported by the IR spectrum^{17, 18} of the compound G, it gave peaks at 1660 cm^{-1} due to $\text{C}=\text{C}$ -stretching vibration and $\text{C}-\text{H}$ bending vibration of double bond at 840 cm^{-1} and 810 cm^{-1} . It was further confirmed by its NMR signals (Fig. III.3) centered at $\delta\ 4.85-5.56$ (multiplet)^{39, 40} for two vinylic protons at C_4 and C_6 . The mass spectrum⁴¹ of the compound gave peaks at $m/e\ 161, 133$ and 107 (Scheme III.A) indicated the presence of two conjugated double bonds at C_4-C_5 and C_6-C_7 respectively.

The presence of phenolic hydroxyl group was shown by peak in the IR spectrum at 3450 cm^{-1} due to $\text{O}-\text{H}$ stretching vibration and 1390 cm^{-1} due to $\text{O}-\text{H}$ bending vibration¹⁷. It was further confirmed by a signal in NMR spectrum concentrated at $\delta\ 12.56$ (singlet)⁴² exchangeable with D_2O . On benzylation it gave a monobenzoate $\text{C}_{21}\text{H}_{29}\text{O}(\text{COC}_6\text{H}_5)$ m.p. $192-94^\circ\text{C}$ and on acetylation, it gave a monoacetate, $\text{C}_{21}\text{H}_{29}\text{O}(\text{COCH}_3)$ m.p. $137-39^\circ\text{C}$, further supported the presence of hydroxyl group in the molecule.

The mass spectrum of the compound showed peaks at $m/e\ 190, 133$ and 107 (base peak) which indicated the presence of a hydroxyl group in the B ring of the sterol at position C_7 .

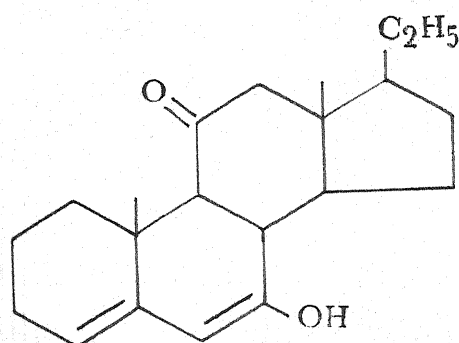
The compound G gave a crystalline yellow solid, m.p. 209-11°C with 2,4-dinitrophenyl hydrazine which responds the presence of ketonic group. The IR spectra also showed the peaks at 1720 cm^{-1} due to C=O stretching vibration for saturated six membered ring ketone⁴¹. It was finally confirmed by mass spectrum which gave characteristic peak at m/e 152. It also confirmed that the ketonic group is in the C ring at position C₁₁.

The point of attachment of side chain in nucleus was C₁₇ which was confirmed by the formation of Diel's hydrocarbon with selenium distillation. Further the side chain was -CH₂CH₃ group which was evidenced by the peak in mass spectrum at m/e 95.

The NMR spectrum of the compound revealed the presence of three methyl groups centred at δ 1.02 (s), for one angular methyl group at C₁₃, δ 1.22 (s) for another angular methyl group at C₁₀, δ 1.75 (s) for terminal methyl group at C₂₀ and δ 1.68- δ 2.90 (m) for methylene groups.

On the basis of the above evidences it can be concluded that the compound G belongs to ketosterols having two conjugated double bonds between C₄-C₅ and C₆-C₇, a phenolic hydroxyl group at position C₇ and a keto group at

position C₁₁. Thus the following tentative structure can be proposed for the compound G.



Compound G

SCHEME III. I. MASS FRAGMENTATION PATTERN OF COMPOUND - G

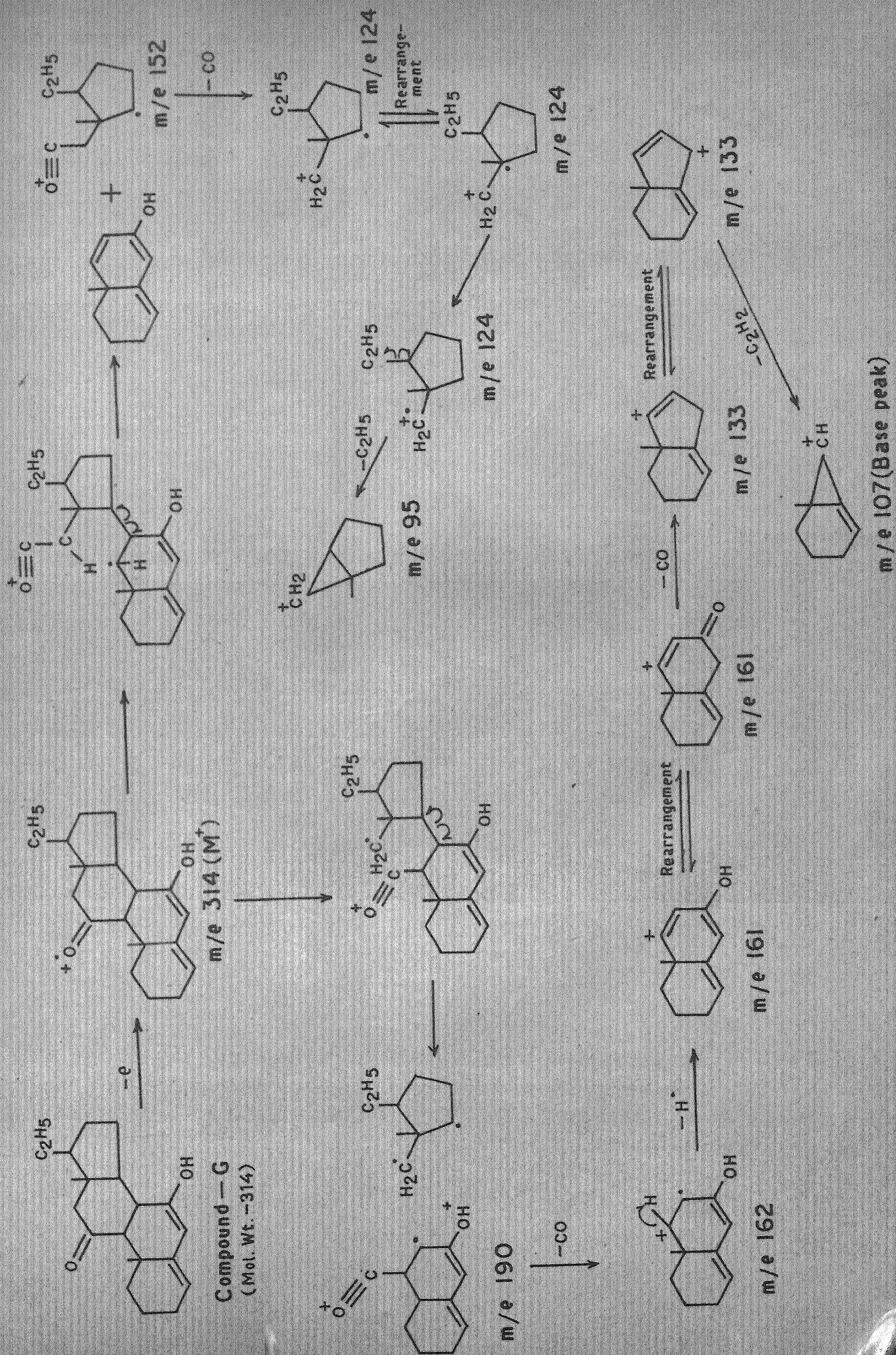
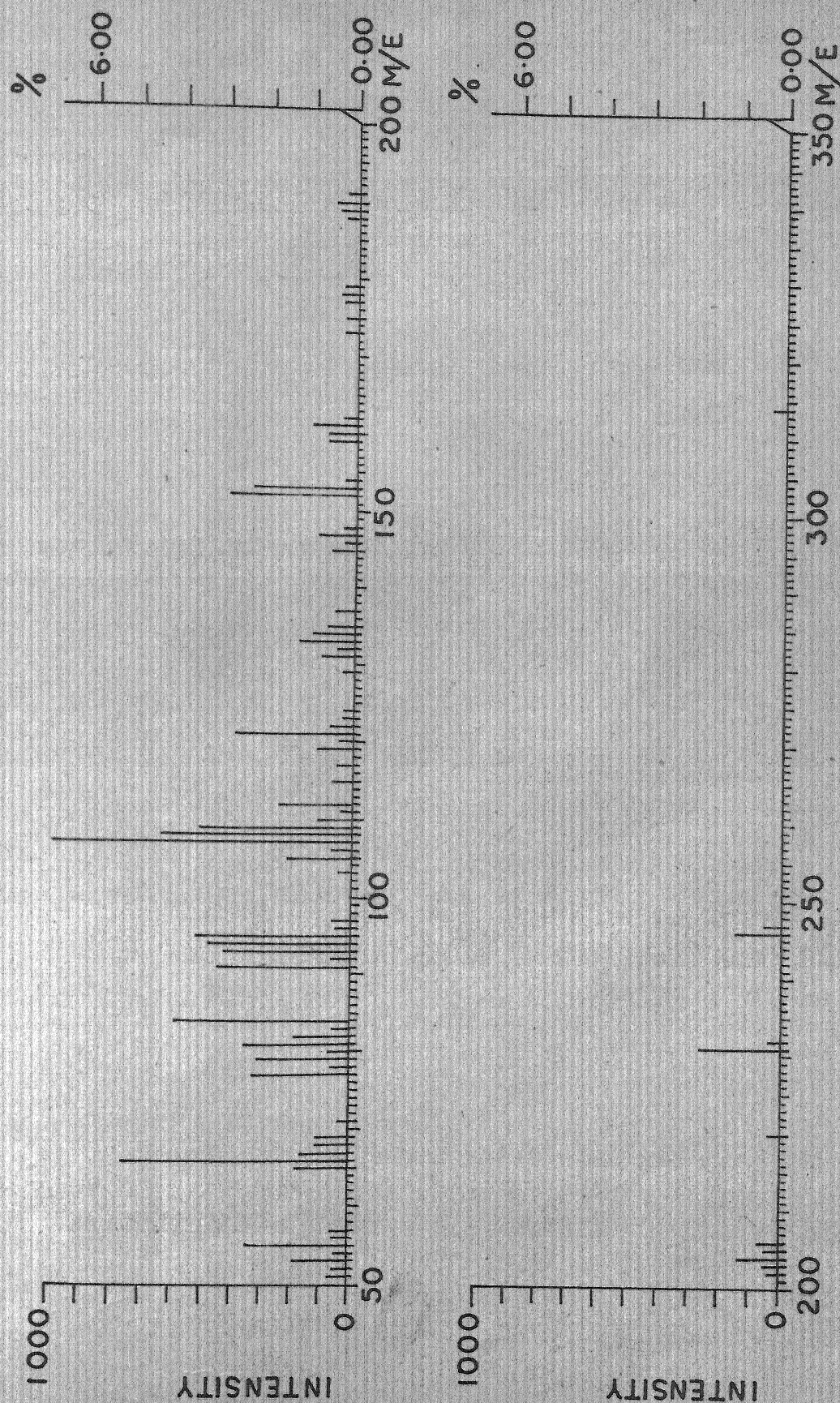


FIG. III. 2. MASS SPECTRUM OF COMPOUND - G



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound G was isolated from the roots of Tinospora cordifolia as described on page 77 . It was recrystallised from hot methanol as colourless crystalline needles, m.p. 183-85°C. The compound was highly soluble in pyridine and hot ethanol and methanol.

The purity of the compound was tested by thin layer chromatography using solvent system, pyridine : methanol (2:5 v/v). The chromatoplate was sprayed with 2N-H₂SO₄ and heated to 110°C for 5 minutes, it gave a single spot of blue colour.

COLOUR REACTION

The compound G gave following reactions for sterols, unsaturation, ketonic group and phenolic group :

1. It gave pink colour changing to red in Liebermann-Burchard reaction.
2. It gave red colour in Salkowski reaction.
3. It gave deep red colour with green fluorescence in Tschugajew reaction.
4. It gave orange colour with Moller's reagent.
5. It decolourised the colour of 1% aqueous potassium permanganate solution or bromine in carbon tetrachloride.
6. It gave white precipitate with maleic anhydride.

7. It gave yellow precipitate with 2:4 dinitrophenyl hydrazine.
8. It gave red colour with neutral ferric chloride.
9. It did not give any colour with ceric ammonium nitrate.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{21}H_{30}O_2$</u>
C = 80.42%	C = 80.26%
H = 9.66%	H = 9.55%
Mol. wt. = 314 (By Mass spectrum)	Mol. wt. = 314

ACETYLATION

Compound (50 mg) were taken in a round bottom flask and mixed 5 ml acetyl chloride and 2 drops pyridine. The reaction mixture was left overnight at room temperature. It was poured in ice cold water with continuous stirring. The precipitate so formed was filtered and crystallised from methanol as white solid, m.p. 137-9°C.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{21}H_{29}O(OCOCH_3)$</u>
C = 77.45%	C = 77.53%
H = 9.17%	H = 8.99%

DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetyl derivative was determined by the method of Wisenberger¹⁹ as described by Belcher and Godbert.

Found	- 12.24%
Calculated for $C_{21}H_{29}O(OCOCH_3)$	- 12.08%

BENZOYLATION

Compound (30 mg) were taken with 1 ml benzoyl chloride and 2 drops of pyridine in a round bottom flask and left for 24 hours. Now reiluxed the mixture for 6 hours on a waterbath. Cooled the reaction mixture and poured in ice cold water containing 2% aqueous sodium bicarbonate. The precipitate so obtained was filtered, washed with 2% sodium bicarbonate solution followed by distilled water and crystallised from methanol. It found to melt at 192-94°C.

BROMINATION

Compound (50 mg) were taken in 5 ml of glacial acetic acid. 3 ml bromine in 10 ml glacial acetic acid was added to the solution with constant stirring. Reaction mixture was kept for 2 hours at room temperature and poured into 100 ml of ice cold water. The solid mass so obtained was filtered and washed with distilled water. It was crystallised from methanol : chloroform (1:1 v/v), m.p. 226-28°C.

SELENIUM DEHYDROGENATION OF THE COMPOUND

The compound was dehydrogenated with metallic selenium in the usual way^{43a}. The product was crystallised from ethanol which melted at 130°C.

2:4 DNPH DERIVATIVE OF THE COMPOUND

The compound (40 mg) were dissolved in 3 ml reagent (prepared according to Fieser and Fieser), warmed and

left overnight at room temperature. The product was filtered and crystallised from methanol, m.p. 209-11°C.

UV SPECTRUM OF THE COMPOUND

UV spectra of the compound was recorded on Perkin Elmer 202 spectrometer using absolute ethanol as solvent. It gave absorption maxima at 222 nm.

IR SPECTRA OF THE COMPOUND

The following prominent peaks in the IR spectrum (KBr) of the compound are obtained. The IR spectra was recorded on Perkin-Elmer infracord.

<u>Position of the peak cm^{-1}</u>	<u>Assignments</u>
3450	-OH stretch vibration
2952	C-H stretching vibration in methyl and methylene group.
1720	C=O stretching vibration due to six membered ring saturated ketone.
1660	-C=C- stretching vibration
1450	-C-H bending vibration in methyl and methylene group
1390	O-H bending vibration
1340	C-H bending vibration
995	-C-C- stretching vibration
840	-C-H bending vibration in double bond
810	

NMR SPECTRA OF THE COMPOUND

A varian A-60D spectrometer was used to record the NMR of the compound, CDCl_3 as solvent and TMS as reference. It gave following main signals :

Signals in δ value

Assignments

1.02 (s)	Angular methyl group at C_{13}
1.22 (s)	Angular methyl group at C_{10}
1.75 (s)	Terminal methyl group at C_{20}
1.88-2.90 (m)	methylene groups
4.85-5.65(m)	Two vinylic protons at C_4 and C_6 .

MASS SPECTRUM OF THE COMPOUND

The mass spectra of the compound was taken on Hitachi RMU-6E spectrometer. It has following main peaks.
 m/e 314, 246, 231, 190, 161, 153, 152, 133, 121, 112, 109, 108, 107, 95, 94, 91, 84, 66.

SECTION - D

STRUCTURAL STUDY OF COMPOUND 'H'

The compound H, m.p. 212-14°C was isolated from the roots of Tinospora cordifolia as described on page 7. The elemental analysis of the compound gave molecular formula $C_{22}H_{32}O_2$. It was soluble in pyridine, hot ethanol and hot methanol.

The compound gave positive colour tests of Liebermann Burchard reaction¹⁰, Salkowski reaction¹¹ and Tschugajew reaction¹². It also gave red-orange colour with Noller's reagent¹⁴ and violet colour in Brieskrone test¹⁵. These reactions indicated the presence of steroids.

It decolourised the colour of bromine in carbon tetrachloride and potassium permanganate solution which is further confirmed by its absorption maxima at 242 nm in UV spectrum³⁸. It gave white crystalline precipitate with maleic anhydride showing the presence of conjugated double bonds. Its IR spectrum^{17, 18} gave peaks at 1650 cm^{-1} due to $\text{C}=\text{C}$ -stretching vibration and $\text{C}-\text{H}$ bending vibration of double bonds at 820 cm^{-1} and 810 cm^{-1} . It was finally confirmed by its NMR signals at δ 5.12-5.68 (multiplet) for two vinylic protons at C_4 and C_6 .

The compound H gave a yellow precipitate with 2,4-dinitrophenyl hydrazine which suggests the presence

of ketonic group. The IR system also showed the peak at 1715 cm^{-1} due to C=O stretching vibration for saturated six membered ring ketone⁴¹.

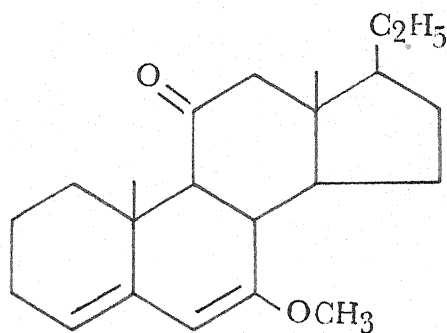
The compound was also found to contain one methoxyl group (Zeisel's method)^{43b} which was confirmed by its IR peaks at 2840 cm^{-1} and NMR signal at $\delta\ 3.91$ (Singlet)⁴¹.

The compound H on demethylation with hydrobromic acid gave a white crystalline solid m.p. $183-5^{\circ}\text{C}$. It gave all the tests for steroids and phenolic group. This demethylated derivative gave an absorption peak at 3430 cm^{-1} and 1390 cm^{-1} for hydroxyl group and it showed no peak in the region of 2840 cm^{-1} for methoxyl group. Finally this derivative was found identical to compound G, already discussed on page 95 in this thesis by m.m.p., co-chromatography and superimposable IR spectra.

It was further confirmed by its NMR spectrum which revealed the presence of three methyl groups at $\delta\ 1.05$ (s) for angular methyl group at C_{13} , $\delta\ 1.35$ (s) for another angular methyl group at C_{10} , $\delta\ 1.75$ (s) for terminal methyl group and at C_{20} and $\delta\ 1.80$, - $\delta\ 3.01$ (m) for methylene groups. (Fig. III.4.)

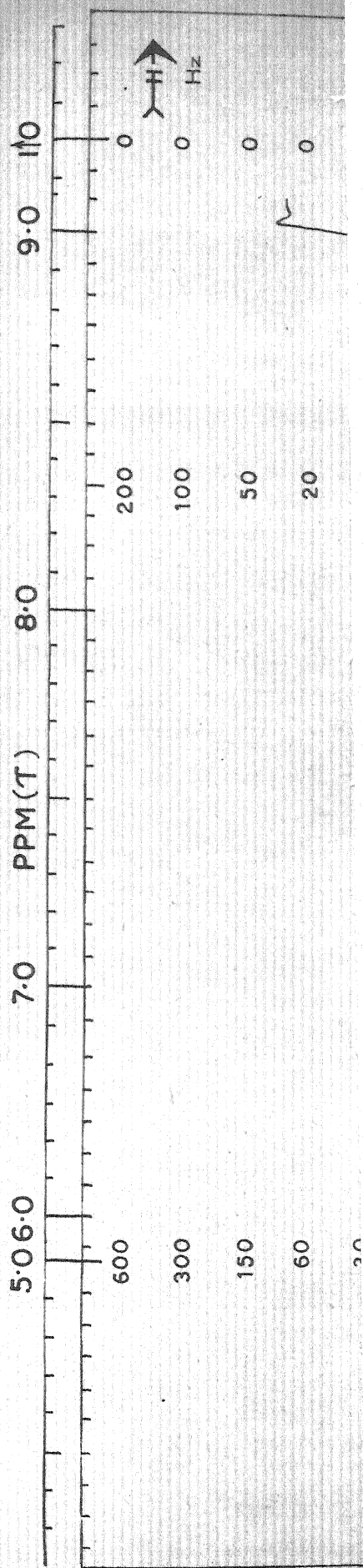
On the basis of the structure of compound G and the above evidences, the compound H may be characterised as the methoxyl derivative of compound G.

Thus its tentative structure may be represented
as follows :



Compound H

FIG. III-4. NMR (CDCl_3) SPECTRUM OF COMPOUND - H



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound H was isolated from the roots of Tinospora cordifolia as described on page 77 . It was recrystallised from hot methanol as colourless crystalline needles, m.p. 212-14°C. The compound was highly soluble in pyridine and hot ethanol and methanol.

The purity of the compound was tested by thin layer chromatography using solvent system pyridine : methanol (3:7 v/v). The chromatoplate was sprayed with 2N-H₂SO₄ and heated to 110°C for 5 minutes, it gave single spot of blue colour.

COLOUR REACTION

The compound H gave following colour reactions for sterols, unsaturation, and ketonic group.

1. It gave red colour in Liebermann-Burchard reaction.
2. It gave red colour in Salkowski reaction.
3. It gave deep red colour with green yellow fluorescence in Tschugajew reaction.
4. It gave red orange colour with Noller's reagent.
5. It decolourised the colour of 1% aqueous potassium permanganate solution or bromine in carbon tetrachloride.
6. It gave white precipitate with maleic anhydride.
7. It gave yellow crystalline precipitate with 2:4 dinitro-phenyl hydrazine.

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound H was isolated from the roots of Tinospora cordifolia as described on page 77 . It was recrystallised from hot methanol as colourless crystalline needles, m.p. 212-14°C. The compound was highly soluble in pyridine and hot ethanol and methanol.

The purity of the compound was tested by thin layer chromatography using solvent system pyridine : methanol (3:7 v/v). The chromatoplate was sprayed with 2N-H₂SO₄ and heated to 110°C for 5 minutes, it gave single spot of blue colour.

COLOUR REACTION

The compound H gave following colour reactions for sterols, unsaturation, and ketonic group.

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6. It gave white precipitate with maleic anhydride.
7. It gave yellow crystalline precipitate with 2:4 dinitro-phenyl hydrazine.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{22}H_{32}O_2$</u>
C = 80.33%	C = 80.49%
H = 9.79%	H = 9.76%
Mol.wt. = 328	Mol.wt. = 328
(By Mass Spectrum)	

DETERMINATION OF METHOXYL GROUP (ZEISEL'S METHOD)

The methoxyl group in the compound was determined by Zeisel's method modified by Belcher, Fildes and Nutton⁶³ as described on page 68 .

<u>Found</u>	<u>Calculated for $C_{22}H_{32}O_2$</u>
Percentage of methoxyl group = 9.48	Percentage of methoxyl group = 9.94

DEMETHYLATION

The compound (50 mg) and 50% hydrobromic acid (10 ml) were refluxed for 1 hour on a water bath. The reaction mixture was diluted with water and filtered. The residue was crystallised from ethanol, into white crystalline solid m.p. 182-84°C.

UV SPECTRUM OF THE COMPOUND

UV spectra of the compound was recorded on Perkin Elmer 202 spectrometer using absolute ethanol as solvent. It gave absorption maxima at 242 nm.

IR SPECTRA OF THE COMPOUND

The following prominent peaks in the IR spectra (KBr) of the compound were obtained which was recorded on Perkin-Elmer infracord.

<u>Position of the peak cm⁻¹</u>	<u>Assignments</u>
2945	C-H stretching in methyl and methylene
2840	Stretching vibration in methoxyl group
1715	C=O stretching vibration due to six membered ring saturated ketone.
1650	-C=C- stretching vibration
1450	-C-H bending vibration in methyl and methylene group
1260	C-OCH ₃ bending vibration
980	C-C stretching vibration
820	C-H bending vibration in double bond.
810	

NMR SPECTRUM OF THE COMPOUND

NMR signals were recorded on a Varian-A-60D in CDCl₃ which gave following main signals.

<u>Signals in δ value</u>	<u>Assignments</u>
1.05 (s)	Angular methyl group at C-13
1.35 (s)	Angular methyl group at C-10
1.75 (s)	Terminal methyl group at C-20
1.80-2.78 (m)	Methylene groups
3.91 (s)	One methoxyl group at C-7
5.12-5.68 (m)	Two vinylic protons at C-4 and C-6.

SECTION -E

STRUCTURAL STUDY OF THE COMPOUND I

A reddish brown coloured compound I, m.p. 152-4°C, has been isolated from ethanolic extract of roots of Tinospora cordifolia as described on page 77 . It was recrystallised from 50% ethanol, R_f value 0.94 in BAW and 0.49 in m Cresol : acetic acid : water (50:2:48 v/v).

The compound gave Molisch test but did not reduce Fehling's solution nor gave a colour with aniline hydrogen phthalate. Thus the compound I is a glycoside.

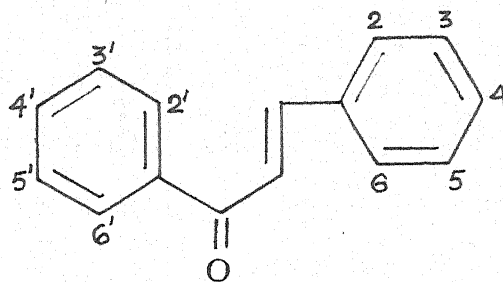
The ethanolic solution of the compound gave following colour reaction of a chalcone-glycoside.

- i) It produced an orange colour with aqueous sodium hydroxide⁴⁴
- ii) It gave no colour when treated with magnesium and hydrochloric acid⁴⁴.
- iii) It produced a light yellow colour when treated with sodium amalgam and concentrated hydrochloric acid⁴⁴.
- iv) It produced a yellow-orange colour when treated with a few drops of concentrated sulphuric acid⁴⁴.
- v) It gave precipitate on treatment with 2,4-dinitro phenyl hydrazine reagent⁴⁵.
- vi) When exposed to vapours of ammonia, on a paper strip, a yellow orange spot of the compound both in visible and UV light⁴⁶.

- vii) On treatment with antimoney trichloride in carbon tetrachloride, a red precipitate was produced^{47,48}.
- viii) A spot of the compound on paper when sprayed with p-toluene sulphuric acid gave orange colour⁴⁶.
- ix) It gave positive test with Vanillin-hydrochloric acid reagent⁴⁹.

These reactions suggest that compound I should be a chalcone derivative. This is further supported by the fact that the compound shows the absorption maxima at 241 nm and two maxima at 312 nm and 334 nm⁵⁰. The IR spectrum of the compound revealed the presence of characteristic peaks at 1630 cm^{-1} for chelated carbonyl and 1608 and 992 for trans configuration $-\text{CH}=\text{CH}-$ confirms the presence of chalcone skeleton.

Thus the compound should have the following basic skeleton :



When the compound was hydrolysed with 7% ethanolic sulphuric acid, it gave a sugar moiety and an aglycone.

The identity of sugar as galactose was confirmed by paper chromatography which revealed a single spot with R_f value 0.18 in n-butanol : acetic acid : water (4:1:5 v/v). Finally it was confirmed by co-chromatography and mixed m.p. with an authentic specimen.

The aglycone obtained by the hydrolysis of the glycosides was red coloured solid m.p. 202°C . It gave some colour reactions (i-ix) as described earlier.

An evanescent green-colour was obtained with ethanolic ferric chloride⁵¹ showing the presence of 2',3' dihydroxy system in aglycone. It was confirmed by the fact that the aglycone showed a bathochromic shift of 78 nm on addition of excess of 1% ethanolic aluminium chloride solution⁵².

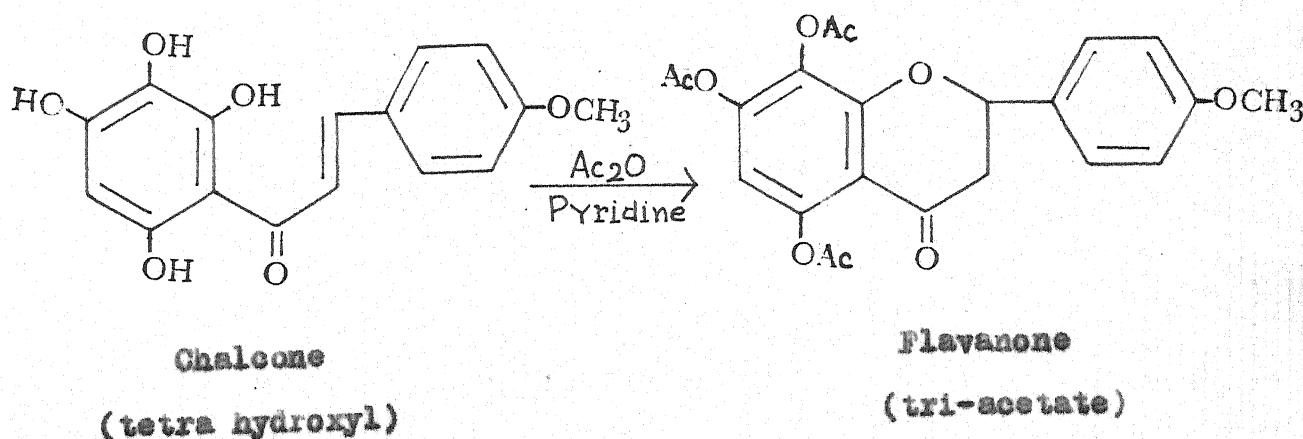
A spot of aglycone on paper when sprayed with p-toluene sulphonic acid⁴⁶ and heated in an oven at 110°C , an orange spot characteristic of a hydroxyl at position 6' is obtained.

The aglycone gave positive test with vanillin-hydrochloric acid reagent⁴⁹, suggesting the presence of 2',4',6' trihydroxy system in the aglycone.

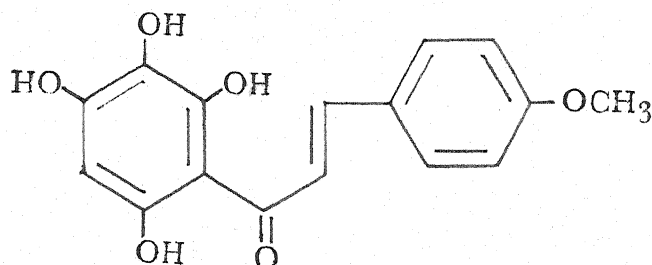
Thus the ring A contains 2',3',4',6' tetrahydroxy system in the aglycone. Finally the alkali cleavage of the aglycone formed 2:6 dihydroxy quinol which confirms the presence of four hydroxyl groups in ring A.

The presence of one methoxyl group was detected by Zeisel's method in aglycone which was confirmed by IR peak at 2820 cm^{-1} ⁵³ since the aglycone gave a bathochromic shift of only 17 nm on addition of sodium ethoxide solution⁵⁴ which shows the presence of methoxyl group at position-4. It was further supported by the fact that the aglycone formed p-anisic acid (p-methoxy benzoic acid) on potassium hydroxide degradation⁵⁵.

On acetylation, the aglycone formed a triacetate, m.p. 155°C , showing the presence of three hydroxyl groups, but it should form a tetraacetate derivative because of the presence of four hydroxyl groups in the compound. This behaviour of the compound has been explained by the fact that a chalcone, during acetylation undergoes an inter-conversion to the isomeric flavanone and instead of tetraacetate (as expected) a triacetate flavanone is obtained⁵⁶⁻⁶⁰.

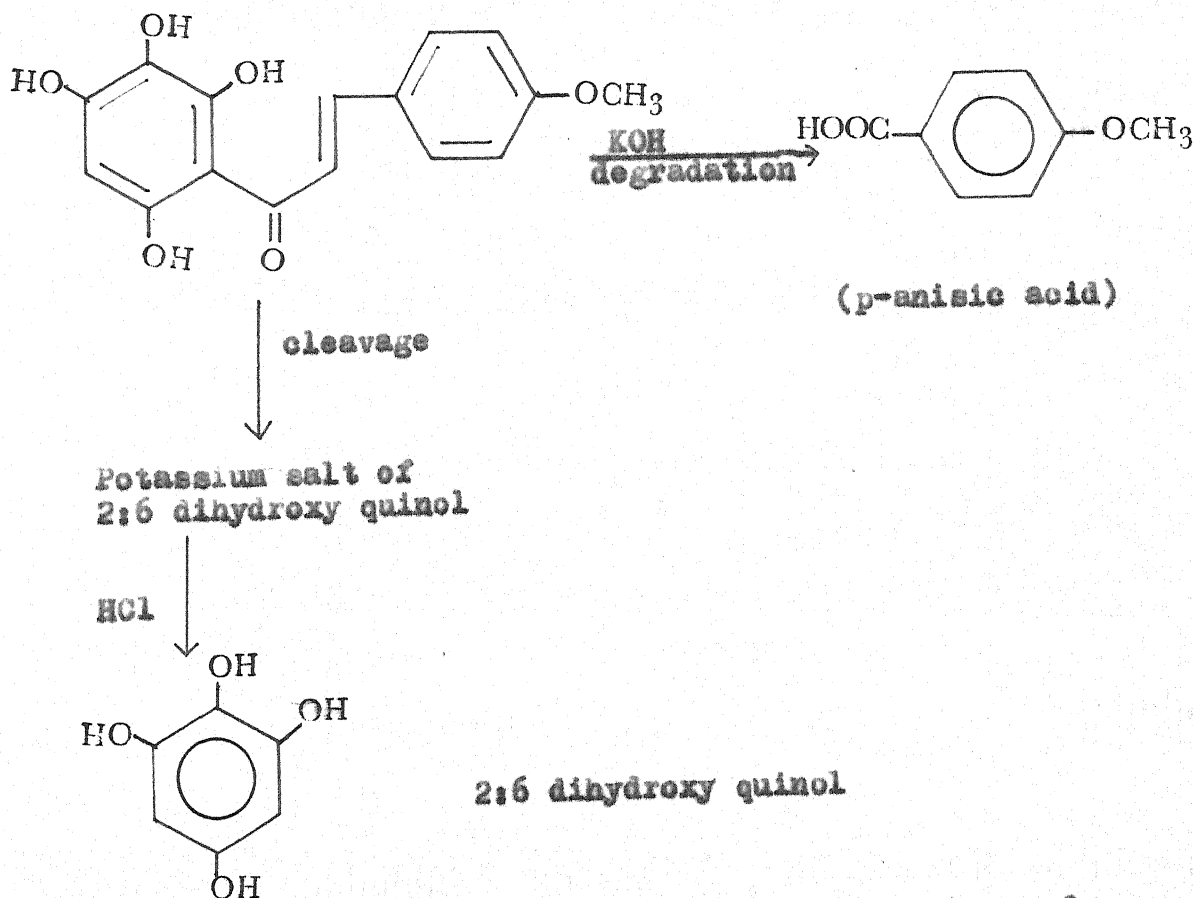


Thus the structure of aglycone is as follows :

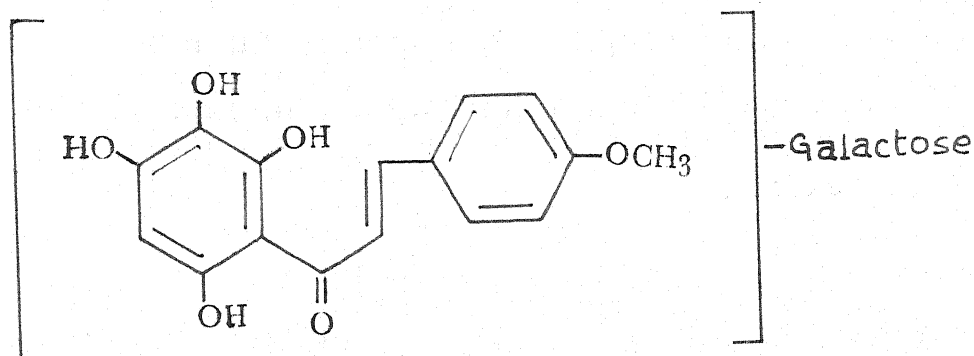


Aglycone

The formation of p-anisic acid and 2:6 dihydroxy quinol as a result of degradation and cleavage of aglycone with alkali may be illustrated as follows :



On the basis of above facts the structure of compound I may be represented as



Now the nature and position of the linkage of sugar moiety have to be decided from its structure. It is obvious that it may be either 2' or 3', or 4' or 6', another point to be decided is whether the sugar linked in the α or β -form.

To ascertain the glycosylation pattern in compound I the following study was made :

1. The glycoside did not give evanescent greenish-blue colour with ethanolic ferric chloride while the aglycone gave it. It shows that the glycoside does not have 2',3' dihydroxy system in the molecule.
2. The glycoside showed a bathochromic shift of 42 nm only on the addition of excess of 1% ethanolic aluminium chloride in the ethanolic solution. The shift⁵⁴ is characteristic for free hydroxyl group at 2'. Thus it is obvious that the galactose should be attached at 3'-position. It is further supported by alkali cleavage of the glycoside which does not give 2',6' dihydroxy quinol.
3. On treatment with a mineral acid, it was found that compound I isomerises into the flavanone derivative thus hydroxyl at 2'-position is free⁶¹ and is not involved in the linkage.

4. Both the glycoside and aglycone show an orange colour with p-toluene sulphonic acid, showing the presence of free hydroxyl at 6'-position.

5. The glycoside and aglycone gave positive test with vanillinhydrochloric acid reagent⁴⁹, suggesting the free hydroxyl group at position 4'.

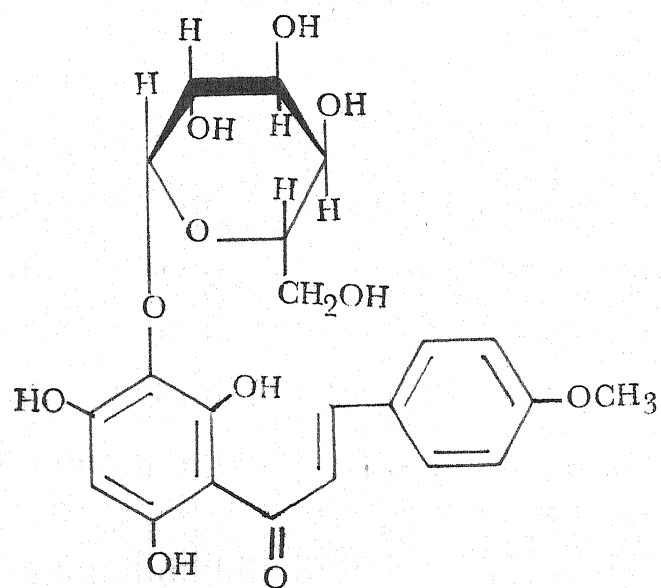
From the above facts it is clear that the hydroxyl groups at position 2',4' and 6' are free and not involved in the glycosidic linkage. Thus it is confirmed that D-galactose is attached with aglycone at position-3'.

On periodate oxidation the compound consumed 2.01 mole of periodate per mole of the compound and 1.12 mole of formic acid was liberated. This indicates that sugar is present in the pyranose form. The presence of medium peaks in the region 845-820 cm^{-1} in IR spectrum of the compound confirmed the pyranose structure of the sugar⁶².

The compound I got hydrolysed with emulsin which is specific for β -linkage. It is further confirmed by the specific rotation $[\alpha]_D^{35} = +32^\circ$ (ethanol) of the compound. Thus the sugar is linked with the aglycone through β -linkage.

Thus the final structure of compound I has been represented as 2',4',6'-trihydroxy 4-methoxy chalcone

3'- β -galactopyranoside.



(Compound I)

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound I was obtained from ethanolic extract of the roots of Tinospora cordifolia as described on page 77. The compound was purified by repeated recrystallisation from 50% ethanol. The purity of the compound was tested by thin layer chromatography using the solvent systems, ethanol ; benzene (75:25 v/v) and acetic acid : formic acid : water (85:6:9 v/v).

THIN LAYER CHROMATOGRAPHY

Descending type of chromatography was done using Whatmann No. 1 filter paper and the following solvent systems:

- i) n-butanol : acetic acid : water (4:1:5 v/v)
- ii) m-Cresol : acetic acid : water (50:2:48 v/v)

The R_f values of the compound I were found 0.94 in solvent system (i) and 0.49 in (ii). The spots were developed by exposing the paper in the vapours of ammonia.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{22}H_{24}O_{11}$</u>
C = 57.52%	C = 56.89%
H = 4.89%	H = 5.17%

HYDROLYSIS OF THE COMPOUND

The compound (500 mg) were hydrolysed with 10% ethanolic sulphuric acid for 10 hours on a boiling water bath. After cooling, the contents were diluted with water and shaken with ether. The aqueous portion was neutralised with barium-carbonate and filtered. The filtrate was concentrated to a syrup.

IDENTIFICATION OF SUGAR

The syrup gave positive Molisch test and reduced Fehling's solution. It was treated with phenyl hydrazine reagent whereupon it formed an osazone, m.p. 194°C and also gave R_f value 0.16 in butanol : acetic acid : water (4:1:5 v/v) system, suggesting the sugar to be galactose. It was further confirmed by co-chromatography, m.m.p. with an authentic sample.

IDENTIFICATION OF AGLYCONE

The ether layer obtained during hydrolysis was washed well and the solvent was evaporated. The solid mass was recrystallised from 50% ethanol as a red coloured compound, m.p. 202°C.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{16}H_{14}O_6$</u>
C = 62.92%	C = 63.57%
H = 4.96%	H = 4.63%

ACETYLATION OF AGLYCONE

Acetylation of the aglycone (30 mg) was carried out as usual described on page 4. The acetylated product was recrystallised from acetone, m.p. 155°.

DETERMINATION OF ACETYL PERCENTAGE

The percentage of acetyl group in acetylated product was determined by the method of Wiesenberger¹⁹ as described by Belcher and Godbert.

<u>Found</u>	<u>Calculated for $C_{16}H_{10}O_6(COCH_3)_3$</u>
Acetyl percentage = 31.24	Acetyl percentage = 30.21

ALKALI DEGRADATION

The alkali degradation of aglycone (200 mg) was carried out as described on page 67. The degradation products were p-anisic acid, m.p. 183°C (lit. m.p. 184°) and 2:6 dihydroxy quinol, m.p. 163°C (lit. m.p. 165°) which were confirmed by co-chromatography and mixed m.p. with the authentic samples respectively.

DETERMINATION OF METHOXYL GROUP

The methoxyl group in aglycone of compound I was determined by Zeisel's method modified by Belcher, Fildes and Nutten⁶³ as described on page 68.

<u>Found</u>	<u>Calculated for $C_{15}H_{11}O_5(OCH_3)$</u>
Percentage of methoxyl group = 10.78	Percentage of methoxyl group = 10.28

PERIODATE OXIDATION

The periodate oxidation of compound I was carried out as described on page 69 .

For each mole of the compound

Moles of periodate consumed = 2.01

Moles of formic acid liberated = 1.12

ABSORPTION SPECTRA

The spectral studies of the compound I were made as described earlier.

<u>Solvents and Reagents</u>	<u>λ_{max} (nm)</u>	<u>Shift (nm)</u>
<u>GLYCOSIDE</u>		
Ethanollic solution of compound	241,334	-
Ethanollic solution + 1% ethanollic $AlCl_3$	376	42
<u>AGLYCONE</u>		
Ethanollic solution of aglycone	348	-
Ethanollic solution + 1% $AlCl_3$	426	78
Ethanollic solution + sodium ethoxide	365	17

IR SPECTRA

The prominent peaks in IR (KBr) spectrum of the compound I were found at

$\nu_{\text{max}}^{\text{KBr}}$: 3300, 3250 (OH), 2910, 2820 (CH and OCH_3), 1630 (C=O), 1608, 992 (-CH=CH), 1575, 1515, 1440 (C-O), 1270 (C-OCH₃), 1205 (C-OH), 1110, 1080, 845, 820.

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CHAPTER - IV

CHEMICAL EXAMINATION OF ECLIPTA ALBA

The plant Eclipta alba, commonly known as Bringraj, belongs to the family 'Compositae'. It is a common weed found in moist environment all over India ascending up to 6000 feet.

The plant of Eclipta alba is a small, coarsely hairy, annual, prostrate herb. Leaves are hairy and variable in shape. Flowers heads white, small, axillary or terminal; involucre of bracts large, bell shaped; outer flowers white, linear, female; inner florets bisexual and tubular.

It is known to be of great importance¹. In Ayurvedic system of medicine, the juice of the whole plant is used as a tonic to cure hepatic and spleen enlargements and in jaundice. Its fresh juice is given in doses of a half to two drachms in fevers, dropsy, liver disorders and rheumatism. Leaf juice along with honey are used as a remedy for Catarrh. It is also given in cough, headache, alopecia and liver disorders. In Burma and Assam natives take leaf juice orally as to darken the hair.

Root is used as purgative and applied externally as an antiseptic to ulcers and mounts in cattle.

The previous work done, on the plant, was surveyed and the details of it are given below :

Constituents	Parts of the plant	References
1. Nicotine	Plant (stems & leaves)	Pal, S.N. and Narasimham, M. ²
2. Wedelolactone	Leave	Govindaachari, T.R., Nagrajan, K. and Puri, B.R. ³
3. Two thiophene derivatives and one polyacetylene	Leaves	Bohlmann, F., Kleine, K.M. and Arndt, C. ⁴
4. α -terthienyl methanol and stigma sterol	Leaves	Krishnaswamy, N.R., Seshadri, T.R. and Sharma, B.R. ⁵
5. Desmethyl wedelo lactone	Leaves	Bhargava, K.K., Krishnaswami, N.R., Seshadri, T.R. and Triuvenkata, P. ⁶
6. Desmethylwedelo lactone and its glycoside, α -ter thienyl methanol, β -amyrin and stigmasterol.	Leaves	Bhargava, K.K., Krishnaswamy, N.R., and Seshadri, T.R. ⁷
7. Weed control	Plant seeds	Ghosh, A.K., Chowdhury, Roy, Sarkar, P.A. ⁸

Literature survey reveals that no work has been done on the roots as well as on stems separately. An attempt has been made in the chapter to study thoroughly for the active chemical constituents from the stems and the roots of the plant.

EXTRACTION AND ISOLATION OF CHEMICAL CONSTITUENTS OF ECLIPTA ALBA

The plants of Eclipta alba were collected and identified for their authenticity in the Botany Department of D.V. College, Orai.

The stems and roots of the dried plants were used separately for extraction.

EXTRACTION OF ROOTS

2 kg of dried and crushed roots were defatted with petroleum ether (60-80°C) in a soxhlet extractor for 20 hours. The extract was concentrated to a yellow coloured waxy mass. The defatted roots were extracted with 95% ethanol in several lots. The combined extracts were concentrated to semisolid mass.

The yellow waxy mass was dissolved in absolute ethanol and added potassium hydroxide (10 gm in minimum volume of water). This mixture was taken in a round bottom flask and refluxed for 2 hours on a water bath. After saponification, the distilled water was added in the ethanolic solution. The excess of ethanol was removed by distillation and the residual portion was extracted with ether. The ether layer was separated from the rest of the aqueous layer.

The ether extract was distilled under reduced pressure when a pale yellow mass was obtained. It was placed to column chromatography on silica gel G. A colourless

solid J was obtained from the petroleum ether : ether (1:4) fraction.

The aqueous layer had insoluble floating material, which was filtered and dried in a vacuum desiccator. This material was then extracted with chloroform and the extract was evaporated to dryness into a cream coloured solid. It was subjected to column chromatography on neutral alumina. A colourless solid K was obtained from the benzene : petroleum ether (1:1) fraction. From ethanol : benzene (1:1) fraction another compound L was obtained.

The semisolid mass obtained from ethanolic extract was refluxed several times with benzene. All the fractions were combined and the solvent was distilled under reduced pressure into a pale yellow semisolid. It was subjected to column chromatography on silica gel G. A colourless crystalline compound M was obtained from benzene : methanol (9:1) fraction.

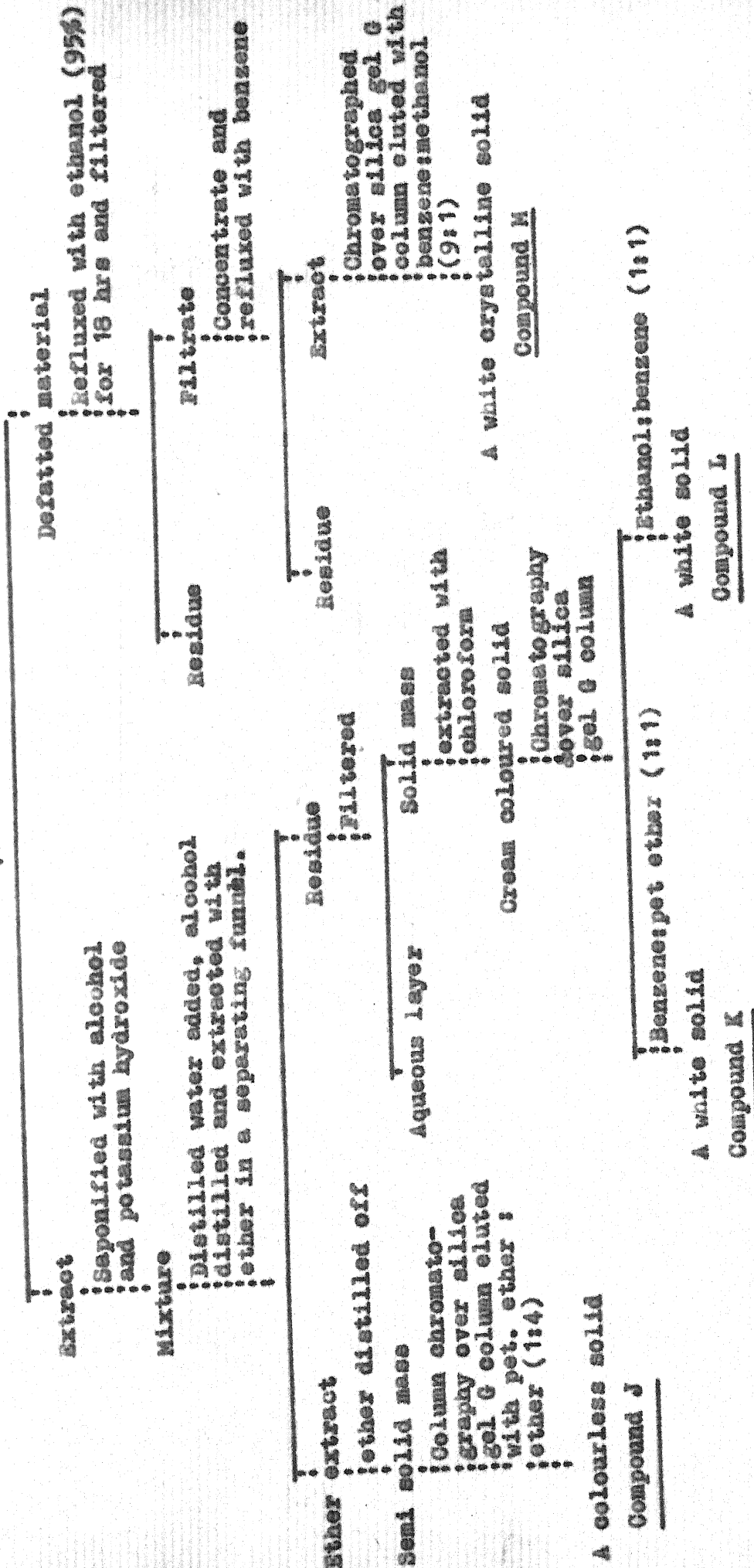
EXTRACTION OF STEMS

Air dried, crushed stems (2 kg) were kept immersed in 1% HCl for 12 hours and then filtered. 20 gm of sodium hydroxide were added to the filtrate and the solution was subjected to steam distillation. The distillation was stopped as soon as the distillate ceased to give pink colour with phenolphthaleine. The aqueous distillate was shaken repeatedly with equal volume of ether in a separating funnel. All

etheral extracts were combined and ether distilled off under reduced pressure. A colourless liquid N remained as a residue. This changed to brown giving tobacco odour.

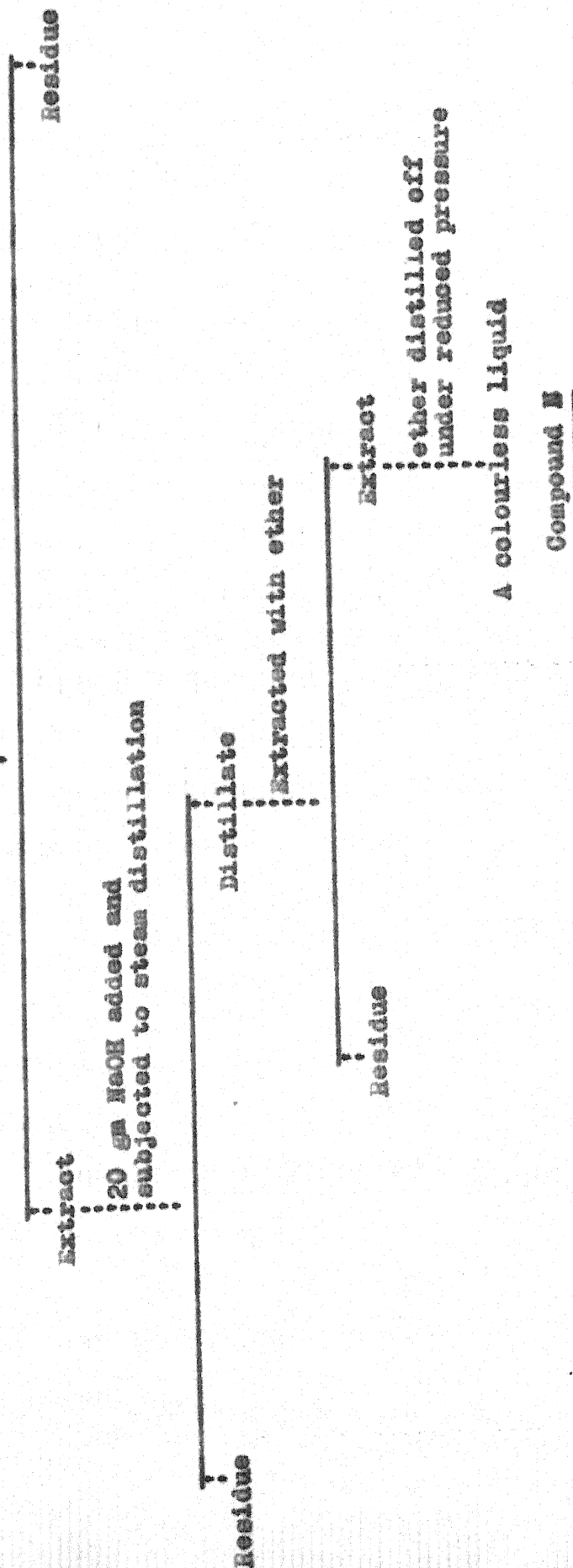
ROOTS OF ECLIPTA ALBA

Defatted with Petroleum ether
(60-80°) in a soxhlet for 20 hrs



STEMS OF ECLIPTA ALBA

.....
Dried and crushed stems were
kept immersed in 1% HCl for
12 hrs and filtered
.....



SECTION - A

CHEMICAL STUDY OF COMPOUND J

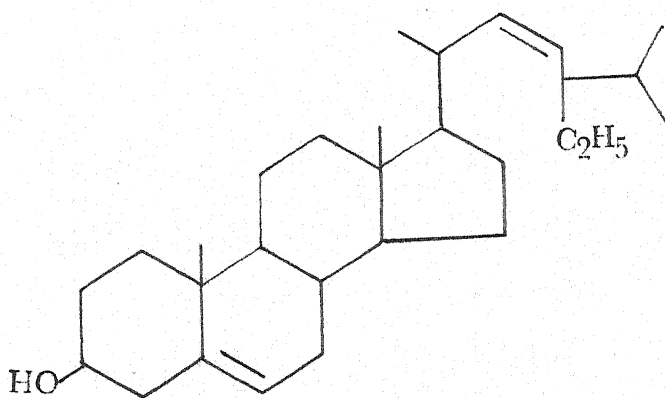
The colourless compound J, m.p. 166-168°C, $[\alpha]_D^{25} - 46^\circ$, was isolated and purified from the roots of Eclipta alba as described on page 130. Elemental analysis of the compound gave molecular formula $C_{29}H_{48}O$, molecular weight was determined by Rast's method - 412. It was soluble in ether, benzene, chloroform, ethyl acetate and ethanol.

The compound J gave Liebermann-Burchard and Salkowski reactions. It also gave colour with Noller's reagent and trichloroacetic acid. These colour reactions are specific for the steroids and terpenoids. Since the compound did not produce any colour by the Brieskrone test⁹, showing the absence of triterpenoids. From the molecular formula and colour reactions, it is evident that the compound J is a sterol. It also gave positive test with tetra nitromethane¹⁰ and decolourised the colour of bromine in carbon tetrachloride and potassium permanganate solution, thereby indicating the presence of olefinic bonds in the molecule. The presence of two olefinic bonds was further confirmed by the formation of tetrabromo derivative, $C_{29}H_{46}OBr_4$, m.p. 194-96°C, and the peaks at 845 cm^{-1} and 705 cm^{-1} ^{11,12} in the IR spectrum of the compound.

It gave a monoacetyl derivative $C_{31}H_{50}O_2$, m.p. $141^\circ C$, $[\alpha]_D - 56^\circ$ and a monobenzoate derivative, $C_{36}H_{52}O_2$, m.p. $158-61^\circ$, $[\alpha]_D - 25^\circ$, indicating the presence of one hydroxyl group. It was further confirmed by the peak at 3510 cm^{-1} , in IR spectrum¹¹ of the compound.

From the above studies it was found that properties of the compound J, its derivatives and IR data (peaks at 3510 , 2950 , 2890 , 1650 , 1460 , 1390 , 1310 , 1255 , 1065 , 1030 , 995 , 845 , 800 and 705 cm^{-1} correspond to stigmasterol^{12,13}. It was finally confirmed by mixed m.p.¹⁴, co-chromatography and superimposition on IR spectrum.

Thus the structure of the compound J (stigmasterol) is as follows :



Compound J
(Stigmasterol)

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound J was isolated from the roots of Eclipta alba as described on page 130 . It was crystallised from 95% ethanol in a colourless plates, molecular formula $C_{29}H_{48}O$, m.p. $166-68^{\circ}C$, $[\alpha]_D - 46^{\circ}$. It was quite soluble in ether, benzene, chloroform, ethyl acetate, methanol and ethanol.

The purity of the compound was tested by thin layer chromatography using the solvent system chloroform : benzene (1:1 v/v). The chromatoplate was sprayed with 5N H_2SO_4 which on heating at $110^{\circ}C$ for five minutes, gave a single spot.

COLOUR TESTS

Compound J showed following colour reactions for sterols :

- a) A deep red colour in Liebermann Burchard reaction.
- b) A yellow colour changing to red in Salkowski reaction.
- c) A red colour on treatment with Moller's reagent.
- d) A violet colour changing to blue with trichloroacetic acid.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{29}H_{48}O$</u>
C = 83.67%	C = 84.40%
H = 11.62%	H = 11.72%
Mol.wt. = 412	Mol.wt. = 412
(By Hast's Method)	

ACETYLATION

The compound was acetylated by the usual method as described on page 50 . The acetylated product was recrystallised from 95% ethanol as white flakes, m.p. 141°C, $[\alpha]_D - 56^\circ$.

BENZOYLATION

The compound was benzoylated in the usual manner as described on page 84 . The benzoylated product was recrystallised from acetone : benzene mixture (1:1) as white plates, m.p. 158-61°C, $[\alpha]_D - 25^\circ$.

BROMINATION

The compound (50 mg) in ether was brominated with brominating mixture (2 ml bromine in 10 ml acetic acid). The mixture was cooled, filtered and washed with ether. It was recrystallised from chloroform : methanol (1:1 v/v), m.p. 194-96°C.

ULTRA VIOLET SPECTRA

A perkin-Elmer 202 spectrometer was used to record ultra violet spectra of the compound in ethanol.

$\lambda_{\text{max}}^{\text{EtOH}}$ - 204 nm log E 3.58

INFRA RED SPECTRUM

The IR (KBr) of the compound was recorded on a Perkin-Elmer infracord. It gave following main peaks in the IR spectrum :

3510, 2950, 2890, 1650, 1460, 1390, 1310, 1255, 1065, 1030, 995, 845, 800 and 705 cm^{-1} .

SECTION - B

STRUCTURAL STUDY OF THE COMPOUND K

A white compound K, m.p. 86°C was isolated from the roots of Eclipta alba as described on page 130. The elemental analysis of the compound gave molecular formula $C_{31}H_{64}O$, it was in agreement with molecular ion peak at m/e 452 in its mass spectrum. It gave pink colour with ceric ammonium nitrate, showing the presence of alcoholic group in the compound.

The compound K did not decolourise potassium permanganate solution or bromine solution, indicating it to be a saturated compound. It did not show any absorption in ultraviolet region and also did not react with 2:4 dinitro-phenyl hydrazine, showing the absence of any chromophoric

C=O group. It has been found to be aliphatic in nature as revealed by the IR absorption bands at 2899 cm^{-1} , 2802 cm^{-1} , 1470 cm^{-1} , 775 cm^{-1} , 722 cm^{-1} 15, 16, 17 (Fig. IV.1).

The infra red spectrum of the compound shows stretching due to polymeric association of hydroxyl group involving in intermolecular hydrogen bonding at 3289 cm^{-1} 18, 19 a peak obtained at 1310 cm^{-1} 20, 21 due to -OH bending vibration of primary alcohols and a peak at 1060 cm^{-1} due to stretching vibration of primary alcohol. These peaks clearly show that the molecule is a primary alcohol²². A peak obtained

at 2899 cm^{-1} ²³ is for C-H stretching and peak obtained at 1470 cm^{-1} is due to $-\text{CH}_2$ bending vibrations^{24,25,26}. A doublet at 775 cm^{-1} and 722 cm^{-1} was indicated that compound has a long chain. This doublet is a characteristic of the compound containing more than four $-\text{CH}_2$ groups in a long straight chain²⁶. The above facts suggested that the compound was a long chain saturated aliphatic primary alcohol.

On acetylation, the compound K formed a monoacetate $\text{C}_{31}\text{H}_{63}(\text{COCH}_3)$, m.p. $69-70^\circ\text{C}$ (Reported $71-72^\circ\text{C}$ ²⁷) and also formed an iodide derivative, m.p. $68-71^\circ\text{C}$ (reported $71.4-72.4^\circ\text{C}$ ²⁸). Thus the formation of acetyl and iodide derivatives confirmed the presence of one hydroxyl group in the molecule.

Further on oxidation with concentrated nitric acid the compound yielded an acid, m.p. 98° , which was found to be a fatty acid by paper chromatography. The elemental analysis of the acid gave molecular formula $\text{C}_{31}\text{H}_{62}\text{O}_2$. These results suggested that the acid was n-hentricontanoic acid which melted at 99°C .

The NMR spectrum of the compound K also confirmed it to be a long chain primary alcohol. The NMR spectrum in CDCl_3 was consistent with that of aliphatic alcohols²⁹. It showed a triplet (3H) at $\delta\ 0.85$ ($J = 5$, cps) due to terminal methyl group^{29,30}. A strong unresolved band at $\delta\ 1.25$ was for protons of $(\text{CH}_2)_n$ group and two protons of C-1 methylene group (near to hydroxyl group) appeared as a triplet at $\delta\ 2.01$

(J = 6 cps). A singlet was obtained for the hydrogen of hydroxyl group at δ 3.64.

Study of mass spectrum showed a molecular ion peaks at m/e 452 besides a prominent molecular ion peak at m/e 434, corresponding to the loss of 18 molecules. It could be explained due to the fact that alcohols are known to undergo dehydration at higher temperature and consequently a prominent peak M-18 appeared at 434. This phenomenon is characteristic of higher alcohols^{29,31,32}. Elimination of water is always followed by the expulsion of ethylene, leading to a peak of substantial intensity at m/e 392. It is a known behaviour for the mass spectra of alcohols possessing more than four carbon atoms³³. At the lower end of mass spectra, besides the peak at 31 due to $\text{CH}_2=\text{CH}^+$, significant peaks of the alkane type are found at m/e 45, 59, 73 etc. resulting from cleavage at C-C bonds. At low mass also some peaks of olefinic type at m/e 72, 71, 58, 57, 44, 43 were found as expected for a primary alcohol. It is a common feature of mass spectra of long chain alcohols^{34,35,36}. At high mass end of spectrum, ions occurred at the interval of fourteen as expected³⁷.

On the basis of the above evidences and the formation of n-hentriacontanoic acid as the oxidation product led to the confirmation of compound K as a straight chain aliphatic alcohol, n-hentriacontanol.



Compound K

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound K was obtained from the roots of Helipta alba described on page 130 . On crystallisation from benzene : acetone (1:2) it gave colourless shining flakes, m.p. 86°C, molecular formula $C_{31}H_{64}O$. The compound was soluble in petroleum ether, chloroform, ether, benzene, acetone and mineral acids. The purity of the compound was tested by thin layer chromatography.

THIN LAYER CHROMATOGRAPHY

Thin layer chromatography was done on silica gel G plates by activating at 100°C, for 15 minutes in an electric oven using the following solvent systems for developing the plates.

- a) Benzene : chloroform (1:9)
- b) Benzene : ethyl acetate (1:1)

A single spot was revealed in both the cases on spraying the plates with 5 NH_2SO_4 .

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{31}H_{64}O$</u>
C = 82.24%	C = 82.21%
H = 14.12%	H = 14.16%
Mol. wt. = 452	Mol. wt. = 452
(By Mass spectrum)	(Calculated)

CHARACTERISTIC REACTIONS

The compound K gave following characteristic reactions:

- a) It was aliphatic in nature.
- b) It did not decolourise bromine solution or potassium permanganate solution.
- c) It did not react with 2,4 dinitrophenyl hydrazine solution.
- d) It did not give any effervescence with aqueous solution of sodium bicarbonate.
- e) When the ethanolic solution of the compound was mixed with few drops of ferric ammonium nitrate dissolved in alcohol, a pink colour was obtained.

ACETYLATION OF THE COMPOUND³⁸

The compound (50 mg) was taken in 250 ml round bottom flask and 15 ml of acetic anhydride and 5 ml of pyridine were added. The mixture was refluxed for 4 hours on an oil bath between 110° to 135°C. The mixture after cooling was poured into a beaker containing ice cold water with continuous stirring. A white product was precipitated which was filtered and washed well with water. It was crystallised from a mixture of chloroform and benzene (1:1). It was dried in a vacuum desiccator and found to melt at 69-70°C.

DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetyl derivative of the compound was determined by the method of Wiesenberger³⁹ as described by Belcher and Godbert⁴⁰.

Found - 8.62%

Calculated for $C_{31}H_{63}O(COCH_3)$ - 8.97%

The percentage of acetyl group corresponds to one acetyl group in the derivative.

IODINE DERIVATIVE OF COMPOUND

The compound (20 mg) was dissolved in 15 ml chloroform and to it 5 ml solution of iodine in chloroform (1 gm dissolved in 10 ml of $CHCl_3$) was added. A small piece of red phosphorus was also added to it as a catalyst.

The mixture was refluxed for two hours on a water bath. It was filtered and kept overnight in a refrigerator. A yellow solid separated out which was filtered and crystallised from petroleum ether (40-60°C) : ethanol (1:1) as colourless needles, m.p. 69°C, corresponded to hexricotanyl iodide.

OXIDATION OF THE COMPOUND

The compound (400 mg) was taken in 50 ml conical flask and 7 ml of concentrated nitric acid was added. The contents of the flasks were refluxed using air condenser,

on a sand bath for 8 hours. The mixture was now poured into a beaker containing 20 ml of ice cold water. This was left for 2 hours and the white deposit, so obtained, was taken in ether. The ether solution was washed two to three times with distilled water to remove adhering mineral acid. The excess of ether was distilled off under reduced pressure. The remaining solid mass was dried in air and crystallised from ethanol as white crystals melting at 98°C . It gave effervescences in the saturated solution of sodium bicarbonate. Thus the compound formed was an acid.

PAPER CHROMATOGRAPHY OF ACID

The paper used in the chromatography was prepared by Ashkey (1955)⁴¹. The Whatmann No. 1 filter paper was washed by descending chromatography with methanol, water, acetone and ether. It was then dried over calcium sulphate in vacuum and passed through ether solution of 12% paraffin oil. This was dried in air and used for chromatography. Following R_f values were obtained in two different solvents.

- a) 0.23 in 75% ethanol
- b) 0.34 in acetic acid : water (9:1)

ELEMENTAL ANALYSIS OF THE ACID

<u>Found</u>	<u>Calculated for $\text{C}_{31}\text{H}_{62}\text{O}_2$</u>
C = 80.09%	H = 79.83%
H = 12.36%	H = 12.20%

IR SPECTRUM OF THE COMPOUND

The compound K gave the following main peaks in the IR spectrum. The spectrum was taken in KBr phase on a Perkin-Elmer infracord spectrophotometer.

<u>Position of the absorption band cm⁻¹</u>	<u>Assignments</u>
3289	-OH stretching due to polymeric association ^{18, 19}
2899 and 2802	C-H stretching ²³
1470	-CH ₂ bending ^{24, 25, 26}
1380	-CH ₃ bending ⁴²
1310	-OH bending vibration ^{20, 21, 43}
1060	-C-O- stretching vibration of primary alcohol ²²
775 and 722	-(CH ₂) _n bending vibration due to straight chain methyl group n > 4 ²⁶ .

NMR SPECTRUM OF THE COMPOUND

The spectrum of the compound K was taken in CDCl₃ using a Varian A-60D spectrometer. Main peaks were obtained as follows :

<u>Signals in δ value</u>	<u>Assignments</u>
0.85 (t) J = 5 cps	3H, terminal methyl group
1.25 (m)	methylene groups.
2.01 (t)	2H of C ₁ methylene group adjacent to hydroxyl group.
3.64 (s)	1H for hydroxyl group.

MASS SPECTRUM OF THE COMPOUND

A Hitachi RMU-6E spectrometer was used to record the mass spectrum of the compound K which gave the following main peaks :

m/e 452, 434, 420, 406, 392, 373, 364, 350, 336, 322, 308, 294, 280, 266, 252, 238, 224, 210, 196, 182, 168, 154, 140, 126, 112, 98, 84, 73, 72, 71, 59, 58, 57, 45, 44, 43, 31.

SECTION - C

STRUCTURAL STUDY OF THE COMPOUND L

A white compound L, m.p. 78-79°C was isolated from the roots of Eclipta alba as described on page 130 . The elemental analysis of the compound gave molecular formula $C_{27}H_{56}O$. It gave pink colour with ceric ammonium nitrate, which showed that the compound is alcoholic in nature.

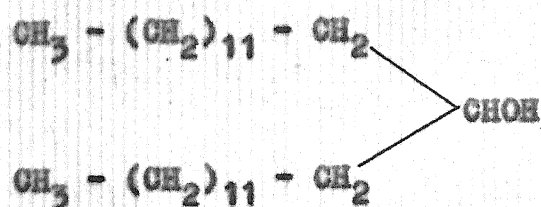
It did not decolourise potassium permanganate solution or bromine solution, indicating it to be a saturated compound. It gave negative test with 2:4 dinitrophenyl hydrazine, showing the absence of carbonyl group.

The infrared spectrum of the compound L (Fig.IV.2) showed stretching vibration of O-H due to polymeric association involving intermolecular hydrogen bonding at 3440 cm^{-1} ^{18,19} and at 4410 cm^{-1} was due to bending vibration of O-H. It is known that O-H bending vibration in alcohols occurs in the region $1400-1300\text{ cm}^{-1}$ ^{20,21}, but the intermolecular hydrogen bonding increases the O-H bending frequency. A peak at 1100 cm^{-1} was due to C-O stretching vibration of secondary alcohol. It is a characteristic of saturated secondary alcohols ²². The peak obtained at 2860 cm^{-1} was due to C-H stretching vibration ²³ and at 1482 cm^{-1} was due to $-\text{CH}_2$ bending vibration. $\text{C}-\text{CH}_3$ bending frequency appeared at

1460 cm^{-1} as a weak peak⁴². A doublet at 765 cm^{-1} and 720 cm^{-1} was due to bending vibrations of $-(\text{CH}_2)_n$ groups in straight chain. The value of n was more than 4²⁶. Thus from the infra red spectrum of the compound L it was confirmed that it was a saturated secondary alcohol.

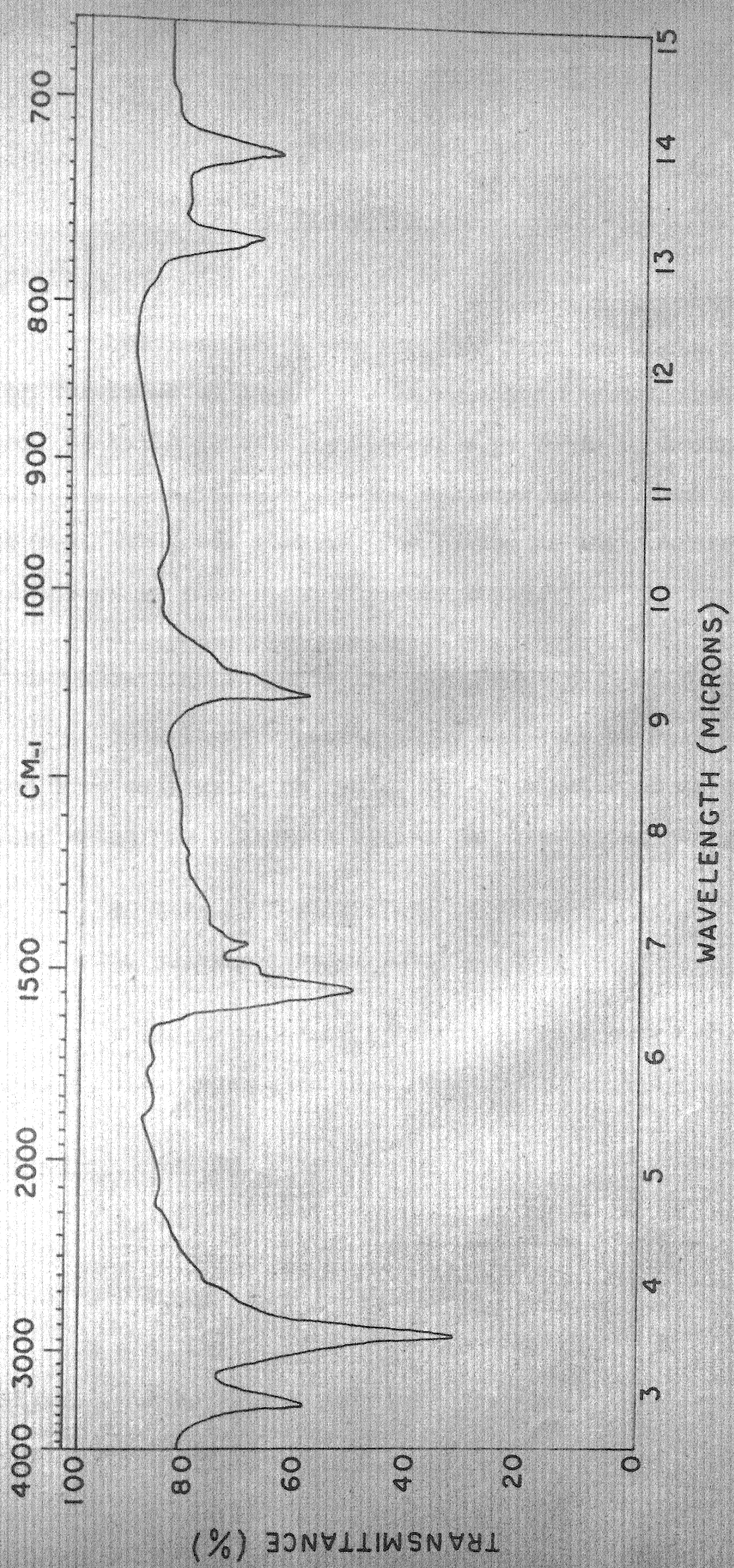
The NMR spectrum and oxidation of compound L could not be done due to paucity of the compound.

The compound gave an acetyl derivative m.p. 44-45°C. A known compound heptacosanol-14, m.p. 80-81°C, formed an acetyl derivative, m.p. 45-45.5°C⁴⁴. The molecular formula of the compound also corresponded to the structure of heptacosanol-14. Therefore, the compound L was identified as heptacosanol-14 which is further confirmed by m.m.p.



Compound L

FIG. IV. 2 INFRARED (KBr) SPECTRUM OF COMPOUND - L



CHARACTERISTIC REACTIONS

The compound L gave the following characteristic reactions :

- a) It was aliphatic in nature.
- b) It did not decolourise bromine solution or potassium permanganate solution.
- c) It did not react with 2:4 dinitrophenyl hydrazine.
- d) It did not give any effervescence with sodium bicarbonate.
- e) It gave pink colour with ceric ammonium nitrate.

ACETYLATION OF THE COMPOUND

The compound L was acetylated in a usual way as described on page 144 . The acetylated derivative was crystallised from ethanol as white crystals, m.p. 45-46°C.

DETERMINATION OF ACETYL PERCENTAGE

The acetyl percentage in the acetyl derivative was determined by the method of Wisenberger³⁹ as described by Belcher and Godbert⁴⁰.

Found - 8.7%

Calculated for $C_{27}H_{55}O.COCH_3$ - 9.8%

The percentage of acetyl group corresponds to one acetyl group in the acetyl derivative.

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The percentage of acetyl group corresponds to one acetyl group in the acetyl derivative.

INFRA RED SPECTRUM

The IR spectrum (KBr) was taken on Perkin-Elmer infracord spectrophotometer. The compound gave following main peaks in the infrared spectrum.

3440, 2860, 1482, 1460, 1410, 1100, 765 and 720 cm^{-1} .

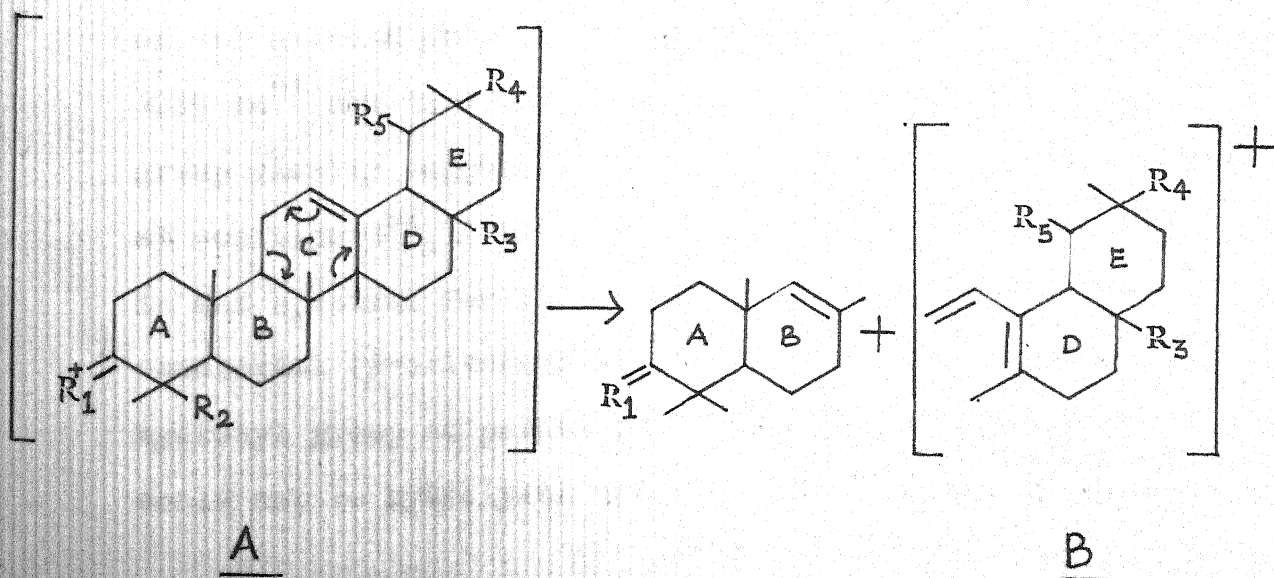
SECTION - D

STRUCTURAL STUDY OF COMPOUND M

The colourless compound M, m.p. 195-197°, $[\alpha]_D + 84.0^\circ$, was isolated and purified from the roots of Eclipta alba as described on page 130. Elemental analysis of the compound gave molecular formula $C_{30}H_{50}O$, this is also in agreement with the molecular ion peak at m/e 426 in its mass spectrum. It responded to colour tests for triterpenoid. The compound seems to be pentacyclic in nature as it gave violet colour with 2,6 di-ter-butyl-p-cresol in ethanol⁴⁵.

It gave a yellow colour (Ruzicka reaction)⁴⁶ with tetranitromethane suggesting the presence of unsaturation in the molecule. It did not show any absorption band above 212 nm in UV spectrum^{47,48,49}, suggesting that the double bond present is not conjugated with the keto group or other double bond present. Since the compound is pentacyclic, it is evident that it must contain the double bond which is sterically hindered, because the compound is not reducible even by Adam's catalyst. The double bond present in the molecule is again supported by the end absorption at 205 nm (log E 3.8) in the UV spectrum. The end absorption at 205 nm (log E 3.8) also indicates the trisubstituted nature of double bond^{50,51,52} which is

further confirmed by one vinylic proton signal in NMR spectrum centered at δ 5.20 (triplet)^{52,53,54}. The IR absorption peaks at 1653, 828 and 818 cm^{-1} , which are characteristic of trisubstituted linkage between the position 12 and 13⁵⁵. This conclusion is further supported by the peaks at m/e 218 (a) and 203 (b) in the mass spectrum of the compound M. Djerassi et al.^{56,57} have established that molecular ion of type (A) undergoes a reverse-Diels-Alder fragmentation to furnish a characteristic ion (B) corresponding to ring D and E. The peak is generally followed by second peak corresponding to (B) minus the C_{17} substituent R_3 . This typical retro-Diels-Alder fragmentation leading to species (B) can thus be employed as a characteristic diagnostic tool for the presence of a 12-13 double bond in triterpene of the oleanone and the ursane series.



The peaks at m/e 218 (a) and 203 (b) (described in scheme No. 1), therefore, clearly suggested

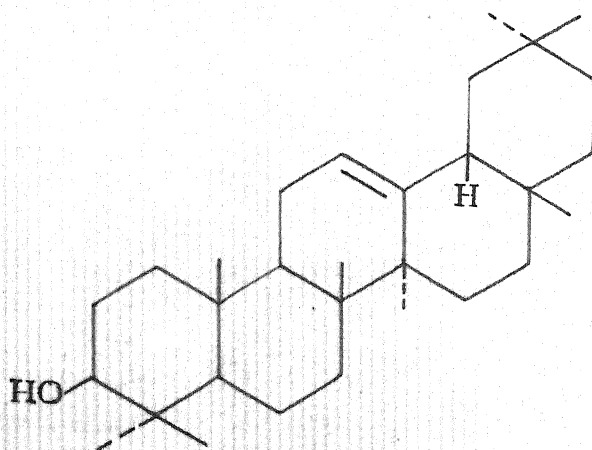
- (i) there is only one double bond between C_{12} and C_{13}
- (ii) there is no substitution in the ring C, D and E and
- (iii) the oxygen atom is either in ring A or in ring B.

The compound had one secondary hydroxyl group as indicated by absorption bands at 3200 cm^{-1} , 1300 cm^{-1} and 1100 cm^{-1} in the infra red spectrum^{5,8} by one proton signal for $3\alpha\text{-H}$ centred at δ 3.28 (triplet⁵³) as well as by the formation of monoacetate, $C_{32}H_{52}O_2$, m.p. $237-40^\circ\text{C}$. On oxidation with chromium trioxide pyridine^{59,60} it gave a product which responded to positive Zimmerman test⁶¹ for β -keto group, thereby suggesting that the hydroxyl group is secondary in nature and it is at the position-3 in the ring A. This is in close conformity with the observation that most of the known number of the pentacyclic triterpenes are oxygenated at C_3 usually as alcohols and the configuration of the natural product is generally β . The IR peaks at 1039 cm^{-1} and 1000 cm^{-1} which are characteristic of β -hydroxyl group clearly suggested the β nature of the hydroxyl group at position-3⁶². A triplet like multiplet centred at δ 3.28 in NMR spectrum for $3\alpha\text{-proton}$ clearly suggested the equatorial (β -orientation)^{53,54} nature of the secondary hydroxyl group at position-3. Further, since the acetate could not be hydrolysed by methanolic sodium carbonate at

room temperature, suggesting the hydroxyl group must be equatorial (β -oriented).

The signals at δ 0.80 (3H), 0.85 (3H), 0.89 (6H), 0.95 (3H), 0.99 (3H), 1.00 (3H) and 1.15 (3H) in the NMR spectrum of the compound revealed the presence of eight tertiary methyl groups. This eliminates the possibility of the compound being related to ursane series^{53,63,64}.

On the basis of the above facts it can be concluded that the compound M belong to oleanane series having a double bond between C₁₂ and C₁₃ and an equatorial (β) hydroxyl group at position-3. Thus the following structure can be proposed for the compound :

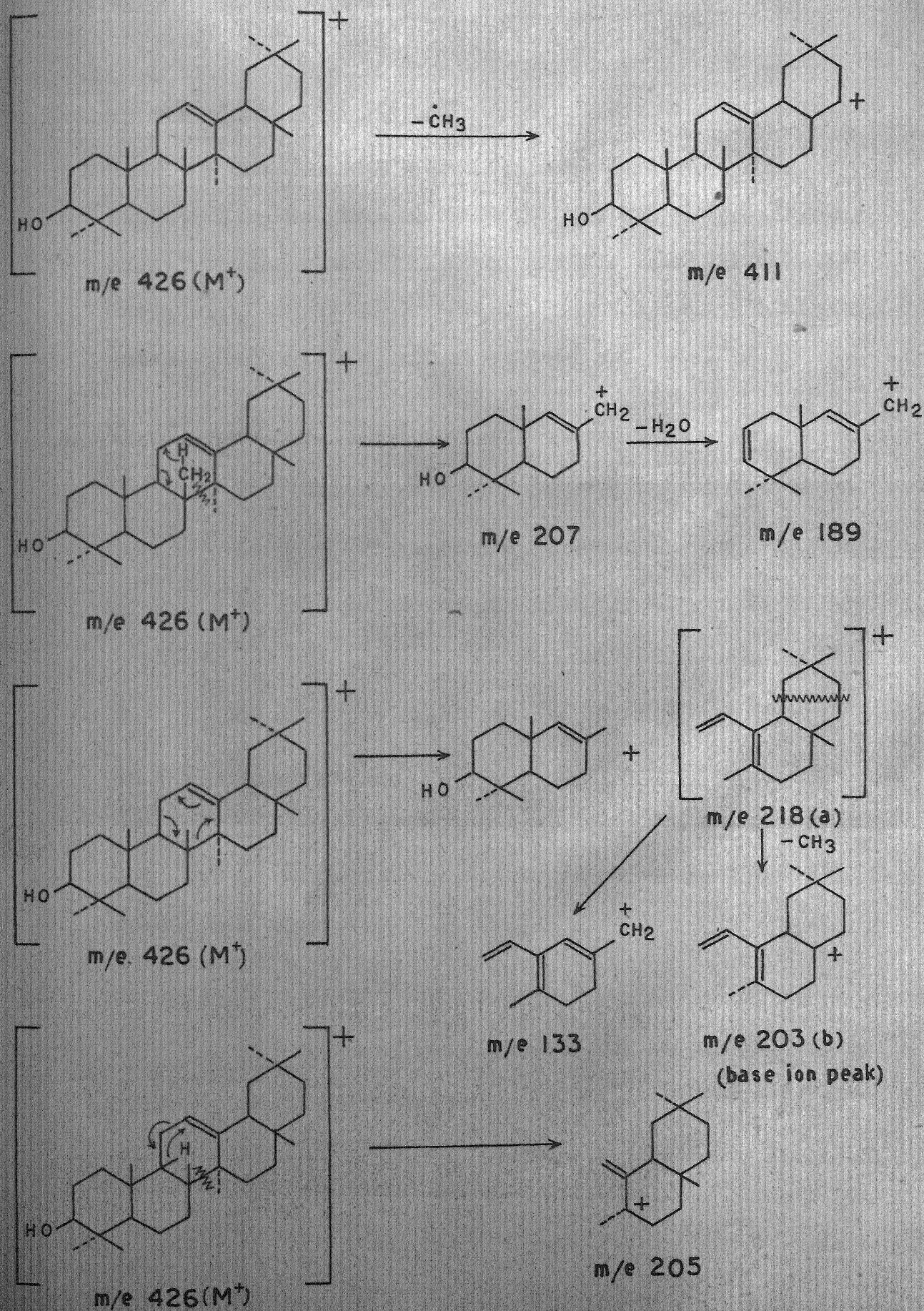


Olean 12-en-3- β -ol

Further, the identity of the compound M as oleon 12-en-3 β -ol (β -anyrin) was confirmed by mixed melting point, co-chromatography and comparing the spectral data^{55,65} and degradative products^{66,67}.

The structure of the compound M was confirmed by mass spectra which showed principal fragmentation peaks at M⁺ m/e 428, 411, 218, 207, 205, 203, 189, 149, 133 (m/e 203-base peak). These fragmentation can be explained on the basis of the above structure^{56,65} (Scheme IV.4).

SCHEME IV. 1. MASS FRAGMENTATION PATTERN OF COMPOUND - M



EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound M was isolated from the roots of Bolipta alba as given on page 130 and was recrystallised from pyridine : methanol (1:4) mixture into white solid mass, m.p. 195-97°C, $[\alpha]_D^{25} + 84.0^\circ$. The compound was highly soluble in pyridine and hot ethanol and methanol.

COLOUR REACTION

The compound M gave following colour reactions :

1. It gave red colour in Liebermann Burchard reaction.
2. It gave yellow colour changing to red in Salkowski reaction.
3. It gave orange red colour in Noller's reaction.
4. Brieskorn test⁴⁵

The compound (2 mg) in ethanol (2 ml) was treated with 2:6 di-tert. butyl p-cresol in ethanol, a violet colour was developed.

5. Ruzicka Reaction⁴⁶

The compound was dissolved in chloroform and to this tetranitromethane in chloroform (1:1) was added, an yellow colour was obtained.

6. Zimmerman test⁵⁵

The oxidised product (2 mg) was dissolved in 1 ml of 2N KOH in absolute ethanol and 1 ml of 1% m-dinitrobenzene in absolute ethanol. After 10 minutes, the mixture was diluted to 10 ml with absolute ethanol. A violet colour developed which faded after some time.

ELEMENTAL ANALYSIS

<u>Found</u>	<u>Calculated for $C_{30}H_{50}O$</u>
C = 84.45%	C = 84.51%
H = 11.75%	H = 11.73%
Mol.wt. = 428	Mol.wt. = 428
(By Mass spectrum)	

ACETYLATION OF THE COMPOUND

The compound (50 mg) in acetic anhydride (5 ml) and pyridine (5 ml) was kept overnight at room temperature. The content was poured into ice cold water. The precipitate was filtered and crystallised from chloroform : methanol mixture to yield an acetyl derivative, $C_{32}H_{52}O_2$, m.p. $237.40^{\circ}C$, $[\alpha]_D = 80.0$.

ELEMENTAL ANALYSIS OF ACETYL DERIVATIVE

<u>Found</u>	<u>Calculated for $C_{32}H_{52}O_2$</u>
C = 82.10%	C = 82.05%
H = 11.14%	H = 11.11%

OXIDATION OF THE COMPOUND

Chromium trioxide and pyridine complex (prepared from CrO_3 (50 mg) and pyridine (1 ml) at 15°C ^{59,60} was added to a solution of compound (50 mg) in pyridine (2 ml) and was allowed to stand overnight at room temperature. Excess of chromic acid was decomposed by the addition of methanol. The mixture was digested with ethyl acetate and on concentration, it gave colourless compound, m.p. $200-203^\circ\text{C}$. It gave positive Zimmerman test for 3 keto groups.

BENZOYLATION

The compound (30 mg) was refluxed with benzoyl chloride (3 ml) and pyridine (5 ml) for one hour. It was poured in ice cold water. The precipitate was filtered and crystallised from a mixture of chloroform and methanol (1:1 v/v) into a crystalline benzoyl derivative, m.p. $233-35^\circ\text{C}$.

INFRA RED SPECTRUM

A Perkin Elmer infracord was used to record the IR spectrum (KBr). The compound gave the following main peaks :

Position of the absorption band cm^{-1}	Assignment
3200 (sh)	O-H stretching vibration ⁵⁸
2900 (s)	C-H stretching in methyl/
2817 (w, shoulder)	of CH_2 ⁶⁹
1470 (s)	C-H bending in methyl/or CH_2 ⁶⁹
1380 (sh)	C(CH_3) ₂ gemdimethyl group resulting from symmetric and asymmetric -O- CH_3 vibrations (triterpene type) ^{70,71}
1355 (sh) doublet	
1110 (sh)	-O-O stretching of secondary alcohol ⁵⁸
1300 (w)	Secondary OH bending vibration ⁵⁸
818 (w)	=C-H bending) Trisubstituted vibration } olefinic type ^{55,58}
828 (m)	
1653 (w, shoulder)	C=C stretching vibration
1200 (w)	C-C- stretching vibration ⁷²
1435 (w, shoulder)	Adjacent - CH_2 to ethylenic double bond ⁵⁵ .
1340 (w)	C-H bending vibration ⁷³
1039 (s)	C-O stretching vibration ⁶² } Due to the β - hydroxyl at position
1027 (w, shoulder)	} ⁷⁴ or due to C-C
1000 (m)	C-C-stretching vibration } stretching or cyclohexane skeleton ⁷²

UV SPECTRUM OF THE COMPOUND

The UV spectra of the compound was run on Perkin-Elmer 202 using ethanol as solvent.

λ_{max} - 205 nm (log E 3.8)

NMR SPECTRUM OF THE COMPOUND

The Varian A-60D spectrometer was used to record the NMR spectrum of the compound which gave following main signals.

<u>Signals in δ value</u>	<u>Assignments</u>
0.80 (s)	(3H) Tertiary methyl group
0.85 (s)	(3H) " " "
0.89 (s)	(3H) " " "
0.95 (s)	(3H) " " "
0.99 (s)	(3H) " " "
1.00 (s)	(3H) " " "
1.15 (s)	(3H) " " "
5.20 (t) $J = 5$ cps	(1H) $>C=CH$ proton at C_{12}
3.28 (m)	(1H) β α -proton.

MASS SPECTRUM

In the mass spectrum of the compound following prominent peaks were observed.

m/e 426 (M^+), 411, 218, 207, 205, 203 (base peak), 189, 149, 133.

SECTION - E

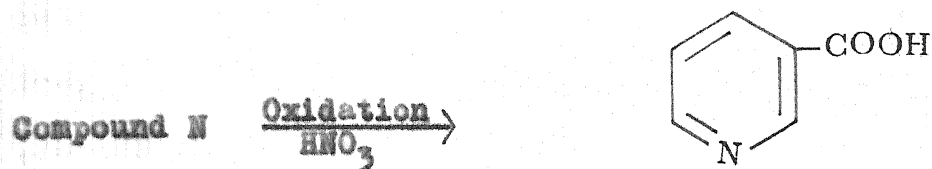
STRUCTURAL STUDY OF THE COMPOUND (B) N

The colourless compound N, b.p. $247-8^{\circ}\text{C}$, $[\alpha]_{\text{D}}^{20} - 168^{\circ}$, was isolated and purified from the stems of Eclipta alba as described on page 131. Elemental analysis of the compound gave molecular formula $\text{C}_{10}\text{H}_{14}\text{N}_2$. On exposure in air it turned brown with tobacco odour. It gave positive test with Wagner's reagent, Mayer's reagent, Chlorohydrin and mercuric chloride reagent for an alkaloid⁷⁵. It gave the absorption maxima at 260 nm and 384 nm in UV spectra, showing that the compound may be a homologue of pyridine.

Further it also gave following specific colour reactions :

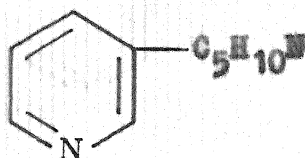
- a) It gave orange colour with epichlorohydrin.
- b) It gave orange red colour with concentrated hydrochloric acid.

On oxidation, it gave a brown solid, m.p. 228°C , molecular formula $\text{C}_6\text{H}_5\text{O}_2\text{N}_2$ which was identified as nicotinic acid⁷⁶ (lit. m.p. 232°) by co-chromatography and m.m.p. with its authentic sample.



Nicotinic acid

From the foregoing discussion it is obvious that the compound N contain a pyridine nucleus with a complex side chain at position-3. Thus the structure of the compound may be written as :



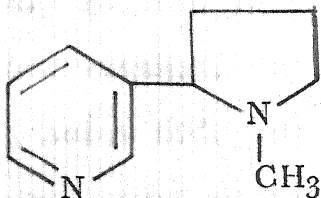
Now with oxalic acid, it gave an oxalate derivative² m.p. 79°C and also formed picrate, m.p. 216-17°C which corresponded to the nicotine oxalate and nicotine picrate (lit. m.p. 218°) respectively.

Further the compound was treated with bromine in hydrobromic acid, it gave a brown coloured crystalline solid, m.p. 192-94°C which corresponded to dibromo ticonine (lit. m.p. 196°)⁷⁷.

These observations shows that the compound may be characterised as nicotine.

It was finally confirmed by the NMR spectra of the compound which showed the signals at δ 2.18 (s) for

methyl group, δ 7.30 (d) for 1H at C₅, δ 7.75 (d) 1H at C-4, δ 8.55 (d) and δ 8.60 (d) for 2H at C₆ and C-2, the protons of pyrrolidine nucleus were concentrated at δ 6.84 and δ 8.21. Comparing the spectral data and degradative products of the compound with those of Nicotine, both were found identical. Thus the compound N was inferred as nicotine.



Compound N

EXPERIMENTAL

ISOLATION AND PURIFICATION

The compound N was isolated from the stems of Eclipta alba, described on page 131. It is a colourless liquid of characteristic odour, b.p. $247.5^{\circ}\text{C}_{\text{D}}$ [α] $^{20}_{\text{D}}$ - 163° , molecular formula $\text{C}_{10}\text{H}_{14}\text{N}_2$. On exposure to air, it turns yellow and eventually brown with tobacco odour. The compound is highly soluble in petroleum ether, solvent ether, benzene and ethanol. It is miscible with water in all proportions, below 60°C and above 210°C but at intermediate temperatures it is immiscible.

CHARACTERISTIC REACTIONS

The compound N gave following characteristic reactions :

- a) A brown flocculent precipitate with Wagner's reagent.
- b) A white precipitate with Mayer's reagent.
- c) A pink colour with phenolphthalein.
- d) Turned red litmus to blue.
- e) A deep red colour with chlorohydrin.
- f) Its ethanolic solution gave orange red colour with epichlorohydrin.
- g) A white crystalline precipitate with mercuric chloride.

- h) An orange red colour with cyanogen bromide and β -naphthylamine.
- i) A light violet colour with concentrated hydrochloric acid which turned to orange with concentrated nitric acid.

ELEMENTAL ANALYSIS

Found

Calculated for $C_{10}H_{14}N_2$

C = 74.8%

C = 74.07%

H = 8.00%

H = 8.67%

N = 16.42%

N = 17.26%

OXIDATION

The compound (50 ml) was taken in a porcelain dish and 5 ml of nitric acid was added and the reaction mixture was left for some time and then evaporated to dryness. A brown solid was obtained, m.p. 228°C , molecular formula $C_6H_5O_2N$. It was identified as nicotinic acid by mixed m.p. and co-chromatography with authentic sample.

OXALATE DERIVATIVE

The compound (30 ml) was dissolved in 2 ml of ether and treated with ethereal solution of calculated quantity of oxalic acid. The reaction mixture was shaken well and the ether was distilled off under pressure. The colourless crystals of nicotine oxalate were obtained, m.p. 79°C .

BROMINATION

The compound (30 ml) was brominated with bromine (2 ml) and hydrobromic acid (5 ml) mixture. The contents were heated on water bath for 2 hrs and poured in ice cold water. A solid mass was precipitated which was filtered, washed and crystallised from methanol as nodular crystals, m.p. 192-94°C.

ULTRAVIOLET SPECTRA

Ultraviolet spectral study of the compound was made in ethanol as a solvent.

$$\lambda_{\text{max}}^{\text{EtOH}} \quad 204 \text{ nm}, 384 \text{ nm}.$$

NMR SPECTRA OF COMPOUND

The compound gave following signals in its NMR spectra which was recorded on a Varian A-60D spectrometer.

δ 2.18 (s, 3H, CH₃); 7.30 (d, d, 1H, C₅-H); 7.75 (d, 1H, C₄-H), 8.55, (d, 1H, C₆-H); 8.60 (d, 1H, C₂-H), 6.84 (m, 4H, C₃-CH₂, C₅-CH₂) and 8.21 (m, 2H, C₄-CH₂).

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CHAPTER - V

The pharmacological studies of some of the isolated compounds have been done in the Department of Pharmacology, G.S.V.M. Medical College, Kanpur and C.D.R.I., Lucknow. The following compounds were examined for their pharmacological activity as described below :

ANTIFERTILITY ACTIVITY

Antifertility activity of compound A (plumbagin), isolated from Plumbago zeylanica was studied. When given orally, it showed significant antiimplantation, abortifacient activity and acute toxicity in albino rats without any tetraogenic effect. It has been reported in the literature that the compound A (plumbagin) has antivulatory effect in rabbits¹.

EXPERIMENTAL

The compound A was examined for its antiimplantation, abortifacient and toxicity separately on albino rats.

ANTIIMPLANTATION ACTIVITY

Adult female albino rats weighing between 125-150 gm showing regular 4-5 days oestrus cycles were divided into 3 groups each containing six rats and housed in separate cages. Young males of known reproductive vigour were kept overnight in the cages in the ratio of 3:1 between females and males.

The day on which spermatazoa were detected in the vaginal smear was termed as first day (D_1) of pregnancy. First group served as controls which were given propylene glycole in the concentration of 1 ml/100 gm body weight. The compound was suspended in propylene glycol for drug solution. The pregnant rats of second and third groups were given different concentrations (1 mg and 2 mg/100 gm body weight respectively) of the drug solution in a constant volume of 1 ml/100 gm body weight. The drug was given daily for five days i.e. D_1 to D_5 of pregnancy.

On the 10th day of pregnancy, the rats were laprotomized and the number of implantation sites were recorded.

With 1 mg dose there was 50% effect and with 2 mg dose the effect was 83.4% (Table I).

ABORTIFACIENT EFFECT

The method employed was the same as described for antiimplantation activity. The only difference was that the various doses of the drug were given orally from the day D_5 to D_{11} of pregnancy. The animals were examined daily for the presence of vaginal bleeding and the day on which bleeding occurred was recorded.

There was 66.6% abortion with 1 mg dose and 83.3% abortion with 2 mg dose. The percentage of abortion

increased as the dose was increased. The occurrence of abortion also became earlier with increasing dose. With 1 mg dose it was seen 11th-13th day of pregnancy while with 2 mg dose abortion occurred as early as on the 8th-10th day of pregnancy (Table II).

TABLE I
ANTIIMPLANTATION EFFECTS OF COMPOUND A IN VARIOUS DOSES FROM
D₁-D₅ OF PREGNANCY IN ALBINO RATS

No. of rats	Drug	Dose/ 100 gm body weight	No. of rats showing implantation on day 10 of pregnancy	No. of rats with no implantation sites	% of antifertility activity
6	Control (Propylene glycol)	1 ml	6	Nil	-
6	Compound A	1 mg	3	3	50%
6	Compound A	2 mg	1	5	83.3%

TABLE II
ABORTIFACIENT ACTIVITY OF VARIOUS DOSES OF COMPOUND A GIVEN
FROM D₅-D₁₁ OF PREGNANCY IN ALBINO RATS

No. of rats	Drugs	Doses mg/100 g body weight	No. of abortion	Day of occurrence of abortion	% of abortion
6	Control (Pyropylene glycol)	1 ml	Nil	-	-
6	Compound A	1 mg	4	11, 12, 12, 13	66.6%
6	Compound A	2 mg	5	8, 8, 9, 9, 10	83.3%

ACUTE TOXICITY²

Study was carried out on 12 adults albino rats of either sex weighing 120-150 gm in four groups of 3 rats each. Group I served as the control and was given propylene glycol in a concentration of 1 ml/100 gm body weight. The rats in group II to IV were given gradual doses of suspension of the compound A in propylene glycol (2 mg, 4 mg and 8 mg/100 gm body weight). The rats were kept under observation for 72 hours and percentage of mortality was also noted.

The compound in dose of 2 mg/100 gm body weight did not produce any toxic effect in rats and no mortality was observed within 72 hours. But with a dose of 4 mg/100 gm body weight, 67% mortality was observed with trouble in respiration. With a dose of 8 mg/100 gm body weight, the toxic symptoms appeared early and the mortality was found 100%.

The above study revealed that the compound A has antifertility effect in rats, if given in a low dose, the antimplantation and abortifacient agent.

ANTI BACTERIAL ACTIVITY

The compound A,B,D,G,H and I were studied for their antibacterial activity. They were screened against two different organisms viz. Escherichia coli and Staphylococcus aureus by agar diffusion techniques³.

EXPERIMENTAL

The nutrient media for the bacteria was prepared as usual by peptone, beef extract and agar-agar as solid nutrient, broth media, at pH 7.4 in a presterilised petri dishes.

The nutrient agar was inoculated aseptically with 0.5 ml of 24 hours old subculture of E. coli and S. aureus in separate conical flasks at 40-45° and mixed well by gentle shaking. About 25 ml of the contents of a flask were evenly spread in each well sterilised petridish and allowed to settle for 2 hrs.

The sterile filter paper discs (5 mm diameter) were saturated with the solution of the test compounds (10 mg/ml in suitable solvents). These discs were placed on the nutrient agar plates after evaporating the solvent. The plates were incubated at the optimum growth temperature of 37° for 24 hours. One experiment was carried in the same condition except the test solution as a control. The zones of inhibition as a diameter of each spot was

measured in mm after 24 hours. The difference of diameter of the zone of inhibition of the test solution and that of the control gives the actual diameter of zone of inhibition of test compound (recorded in table III). Phenol solution 5% was used as reference antibacterial.

TABLE III

SCREENING OF COMPOUNDS FOR ANTIBACTERIAL ACTIVITY

S. No.	Compound	Antibacterial activity	
		E. coli	S. aureus
1.	Control	+++	+++
2.	Compound A	-	-
3.	Compound B	+	-
4.	Compound D	+	+
5.	Compound C	++	++
6.	Compound E	++	+++
7.	Compound I	+	+

- = No inhibition
- + = Zone size 5-8 mm
- ++ = Zone size 8-10 mm
- +++ = Zone size greater than 10 mm.

The results (Table III) shows that all the compounds were found to exhibit antibacterial activity against one or other type of bacteria. Compound A and B

have remarkable activity while compound G and H have least activity.

FUNGICIDAL ACTIVITY

Compounds A,B,D,G,H and I were also tested for their fungicidal activity⁴ and found to exhibit a good fungicidal activity. Two species of fungi were used in the present study :

1. *Aspergillus niger*
2. *Alternaria tenuis*

EXPERIMENTAL

Sabouraud's broth (peptone 10.0 gm/litre, dextrose 20.0 gm/litre, pH 6.0 approx.) was used as the test medium. The compounds were dissolved in ethanol (100 ug/ml) to use it as test solution. The 1.0 ml of the medium was taken in each sterilised tube and 1.0 ml of test solution (100 ug/ml) was added to it by means of sterile micro pipette. Tubes were autoclaved at 15 lbs for 15 minutes, prior to inoculation with suitable species of fungus. After inoculation, tubes were incubated at $26^{\circ} \pm 2^{\circ}$ for 7 days and the vegetative growth recorded visually in each case (Table IV). One experiment was carried out under the same condition except the addition of test solution as a control.

TABLE IV
SCREENING OF COMPOUNDS FOR ANTIFUNGAL ACTIVITY

S. No.	Compounds	A. niger	A. tenuis
1.	Control	+++	+++
2.	Compound A	++	++
3.	Compound B	++	+++
4.	Compound D	+	+
5.	Compound G	+++	+++
6.	Compound H	+++	+++
7.	Compound I	-	+

+++ = Good growth and no fungicidal activity.

++ = Moderate growth and very slight fungicidal activity.

+ = Slight growth, slight fungicidal activity.

- = No growth, fungicidal activity.

The results (Table IV) show that compound D and I show good fungicidal activity while the other compounds do not show satisfactory activity.

The compound N (Nicotine) is a well known alkaloid. It is a very toxic in nature and a fatal dose for man is 40 mg. On the other hand its derivative, Nicotinic acid, has a great medicinal value⁵. It has vasodilator effect, producing sensation of heat and itching.

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